UNIVERSITÉ DU QUÉBEC À MONTRÉAL

TEMPERAMENT ET SYNDROMES COMPORTEMENTAUX DANS UNE POPULATION SAUVAGE DE TAMIAS RAYÉS, *TAMIAS STRIATUS*

MÉMOIRE

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AVANT-PROPOS

Nous avons choisi de présenter ce mémoire de maîtrise sous la forme de deux articles scientifiques. Le premier article sera soumis sous le titre de "Temperament and habituation to novelty: a field study in a wild population of Eastern chipmunks (*Tamia striatus*)" et le deuxième article portera le titre "Animal temperament, reaction to human disturbance, and consequences for the study of stress response of wildlife to tourism exposure". Pour les deux articles, les auteurs seront Julien Martin (UQAM) et Denis Réale (UQAM). La rédaction ainsi que les analyses des résultats obtenus pour l'élaboration des deux présents articles scientifiques ont été effectués par Julien Martin sous la supervision de Denis Réale.

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BLUP	Best Linear Unbiased Predictors
e.g.	par exemple (latin : *exempli gratia*)
DF	Degree of Freedom
EIA	Enzyme ImmunoAssay
et al.	et autres (latin : *et alii*)
Fig.	Figure
H.D.	Head-dipping
ID	Identity
i.e.	c'est-à-dire (latin : *id est*)
ex.	Exemple
K	Number of parameter
Loc.	Locomotion
Loglik	Log-likelihood
LR	Log-likelihood Ratio
LRT	Log-likelihood Ratio Test
m	Mètre
mg	Milligramme (10^{-3} gramme)
min	minute
ml	Millilitre
PBS	Phosphate Buffer Solution
PC	Principal Component
PCA	Principal Component Analysis
pers. obs.	personal observation
pg	Pico-gramme (10^{-12} gramme)
REML	Restricted Maximum Likelihood
rpm	rotation per minute

RÉSUMÉ

Depuis ces dernières années, un nombre croissant d'études se sont intéressées à la variabilité interindividuelle et à la consistance individuelle des comportements et explorent les implications écologiques et évolutives de cette variation. En fonction de l'approche utilisée, la variation individuelle des comportements a été appelée débrouillardise, tempérament, personnalité ou encore syndrome comportemental. Une étape importante dans le développement d'une approche évolutive et écologique de l'étude du tempérament est le développement de méthodes appropriées pour réaliser des mesures de tempérament directement sur le terrain. L'acquisition de connaissances et une meilleure compréhension des variations de tempérament dans des populations animales pourraient avoir un impact important en écologie et en conservation. Les deux premiers objectifs de cette étude étaient de mettre au point un moyen de mesurer des traits de tempérament dans une population sauvage de tamias rayés (*Tamias striatus*) en répétant directement sur le terrain des tests de la planche à trous sur chaque individu et d'explorer l'utilisation des modèles mixtes pour analyser la variation individuelle des comportements et de l'habituation aux tests expérimentaux. Nous avons mis en évidence une consistance individuelle pour l'exploration, l'émotivité et la docilité, ainsi qu'un transfert individuel de comportement avec un gradient de nouveauté. Le troisième objectif était de voir l'effet des variations de tempérament sur la distribution spatiale des individus en réponse à la perturbation humaine. L'étude s'étant déroulée dans une zone fortement fréquentée par les humains, nous nous sommes intéressés à l'effet de la perturbation humaine sur la réponse au stress des tamias (concentration de cortisol). Les tamias les plus explorateurs et les moins farouches étaient localisés dans les zones de perturbation humaine élevée. De plus, seul le tempérament affectait les taux de cortisol. La répartition non-aléatoire du tempérament selon la perturbation pourrait se traduire par la mise en évidence de différences comportementales et hormonales entre des individus localisés sur des zones perturbés et non-perturbés, différences qui ne seraient pas dues à la perturbation. L'intégration de mesures du tempérament pourrait donc avoir un effet important sur l'estimation de l'impact des activités humaines sur la faune sauvage.

Mots-clés : tamias rayés, tempérament, syndrome comportemental, test du holeboard, cortisol.

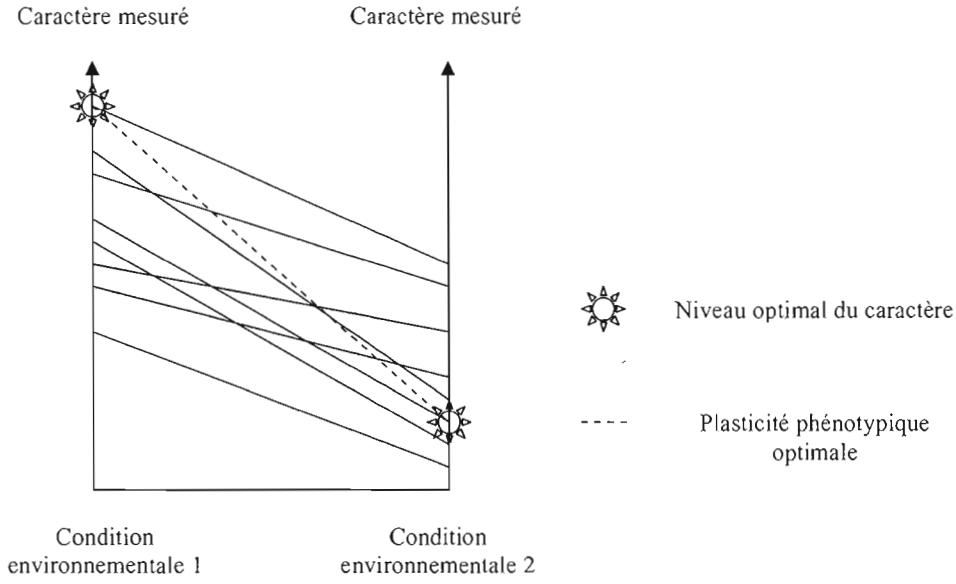
INTRODUCTION GÉNÉRALE

L'approche évolutive principalement utilisée par l'écologie comportementale consiste à expliquer et à comprendre la distribution des comportements contemporains au regard des pressions de sélection passées et de l'historique d'une population (Sih *et al.*, 2004). La sélection naturelle et la sélection sexuelle sont des composantes majeures dans l'explication de la distribution des caractéristiques des êtres vivants contemporains. En ne conservant que les caractères des individus les mieux adaptés aux conditions du milieu, ces pressions aboutissent au maintien d'un caractère optimal, commun à l'ensemble de la population vivant dans ce milieu (Kramer, 2001). De ce fait, elles exercent une force érosive sur la variation génétique et phénotypique d'un trait dans une population. Cette approche, appelée théorie de l'optimalité, prédit que les caractères phénotypiques possèdent un optimum dans des conditions environnementales données. Suite à l'effet de la sélection sur le long terme, ces caractères devraient être à leur optimum (la valeur moyenne d'un caractère dans la population devrait coïncider avec cet optimum).

Selon cette théorie, l'optimal d'un caractère pourrait également changer en fonction des conditions du milieu (Dewitt, Sih & Wilson, 1997). Si la population a évolué dans un environnement variable, l'individu optimal devrait pouvoir changer son comportement instantanément de façon à se positionner au niveau de réponse optimale correspondant à chaque condition spécifique ; un tel individu posséderait une forte plasticité phénotypique (Dewitt, Sih & Wilson, 1997; Kramer, 2001). Ce genre de changement phénotypique, comportemental ou physiologique, peut s'observer à l'échelle de la vie d'un individu (Clark & Ehlinger, 1987). A titre d'exemple, un organisme devrait ajuster son comportement exploratoire au niveau de prédation, car le niveau d'exploration optimal varie selon le risque de prédation. En

explorant son habitat, l'individu peut bénéficier de la découverte de nouvelles parcelles de nourriture, de nouveaux partenaires sexuels, ainsi que de nouveaux abris contre les prédateurs (Heinrich, 1995; Mettke-Hoffmann, Winkler & Leisler, 2002). Il peut également découvrir un nouvel habitat de meilleure qualité que son habitat d'origine. En revanche, l'exploration augmente les risques de rencontre d'un individu avec ses prédateurs (Greenberg & Mettke-Hoffmann, 2001). Nous devrions nous attendre à ce que les individus de populations soumises depuis longtemps à de forte pressions de prédation soient moins explorateurs que celles qui ont eu peu de prédateurs (Mettke, 1995). De plus, un individu devrait diminuer ses comportements exploratoires lors d'épisodes de forte prédation et les accroître lorsque les risques de prédation sont faibles. Selon cette hypothèse, la plasticité phénotypique d'un individu ne serait en aucun cas contrainte et tous les individus seraient semblables dans leur réponse. Pourtant, dans de nombreux cas (Riechert & Hedrick, 1993; Sih *et al.*, 2004), les individus (ou génotypes) varient dans leur réponse et ceux dont le phénotype est proche de l'optimum dans une condition de milieu montrent un phénotype éloigné de l'optimum dans d'autres conditions (figure I.1).

Un nombre croissant d'études sur le comportement animal se sont intéressées récemment à la variation individuelle et à la corrélation entre comportements dans des populations sauvages (poissons : Coleman & Wilson, 1998; souris et rats : Crusio, 2001; File, 2001 ; oiseaux : Greenberg & Mettke-Hoffmann, 2001; Heiblum *et al.*, 1998; Koolhass *et al.*, 1999; pieuvre : Mather & Anderson, 1993; araignées : Riechert & Hedrick, 1993; Sih, Kats & Maurer, 2003; Veenema *et al.*, 2003; Verbeek, Drent & Wiepkema, 1994; Wilson *et al.*, 1994; Wilson *et al.*, 1993). En fonction de l'approche utilisée, la variation individuelle d'un comportement a été appelée débrouillardise (Koolhass *et al.*, 1999), tempérament (Buss & Greiling, 1999), personnalité (Gosling, 2001) ou encore syndrome comportemental (Sih *et al.*, 2004).



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Un syndrome comportemental est une suite de traits comportementaux ou/et de traits hormonaux corrélés (Clark & Ehlinger, 1987; Sih *et al.*, 2004). Les corrélations mises en évidence entre comportements sont généralement phénotypiques, et leurs causes supposées sont génétiques. Cette corrélation serait due à l'existence de mécanismes communs (génétiques et neuro-physiologiques) impliqués dans l'expression de chaque comportement, ce qui limiterait une évolution indépendante de chaque trait (Riechert & Hedrick, 1993; Sih, Kats & Maurer, 2003). Ainsi, du fait de leur lien génétique, un comportement peut changer suite à une sélection sur le comportement qui lui est associé génétiquement, même s'il n'est pas lui-même l'objet de sélection. Ces mécanismes d'expression communs proviennent de ce qu'un ensemble de gènes affecte les réactions cellulaires (ex : synthèse d'ARN messagers et de protéines à partir de gènes), puis physiologiques (ex : régulation hormonale), et finalement l'expression de chacun des comportements impliqués dans le syndrome (Johnston & Edwards, 2002). L'étude des corrélations comportementales a des implications importantes pour l'écologie; elle peut permettre de comprendre l'existence de comportements qui semblaient non optimaux. Par exemple, la forte activité de certaines larves de salamandre (*Ambystoma barbouri*) en présence de prédateur n'est pas considérée comme adaptative (Sih, Kats & Maurer, 2003). L'étude de ces corrélations permet également une meilleure compréhension des comportements d'un animal dans différentes conditions environnementales. Ainsi, ces comportements peuvent s'exprimer dans un contexte environnemental donné ; par exemple, dans un contexte de prédation, les individus présentant une activité élevée en absence de prédateurs sont aussi les plus actifs en leur présence. Les comportements peuvent également s'exprimer à travers différents contextes (corrélations entre des comportements de prise alimentaire, anti-prédateur, accouplement, agressivité, ou dispersion). Lorsque les comportements corrélés subissent des pressions de sélection opposées, leurs expressions atteignent un équilibre qui se traduit dans la limitation de la plasticité de ces comportements (Sih *et al.*, 2004). Sih et ses collaborateurs(Sih, Bell & Johnson, 2004; Sih *et al.*, 2004),

introduisent le concept de **consistance individuelle inter-environnementale** («**individual carryover**») pour décrire la plasticité limitée d'un individu. Ainsi, le maintien de variabilité de l'activité chez la salamandre peut être expliqué par une plasticité phénotypique limitée de ce comportement, et par des niveaux optimaux d'activité opposés en présence et en absence de prédation (Sih *et al.*, 2004).

Selon Wilson *et al.* (1994) et Coleman *et al.* (1998), le « **tempérament** » peut être défini comme la réaction comportementale d'un individu à une situation nouvelle ou risquée. Wilson *et al.* (1994) proposent une répartition continue des différentes réactions face à une situation stressante, selon un continuum timide-hardis (shy-bold continuum). D'une manière plus générale, le tempérament a été défini comme l'ensemble des différences comportementales interindividuelles consistantes à travers le temps et les situations, et qui résultent d'une influence combinée génétique, épigénétique et environnementale (Reale & Festa-Bianchet, 2003; Reale *et al.*, 2000). Par ailleurs la personnalité est définie comme un ensemble de comportements caractéristique d'un individu et qui est consistant à travers le temps et les situations (Gosling, 2001). La différence entre la personnalité et le tempérament est assez vague et semble arbitraire. Ces termes peuvent être considérés comme des synonymes (Réale *et al.*, unpublished; Gosling, 1998). Le concept de tempérament est aussi proche de celui de syndrome comportemental. Cependant, la notion de syndrome comportemental est plus générale puisqu'elle prend en compte n'importe quel type de comportement que ce soit un trait de tempérament ou non. Un trait de tempérament est un ensemble de variables mesurables (comportementale ou physiologique) corrélées associées à l'activité, la réactivité, l'émotivité, la sociabilité et l'agressivité des individus (Buss *et al.*, 1987; Réale *et al.*, unpublished).

Koolhass *et al.* (1999) proposent le concept de **débrouillardise** (ou **coping**) : un effort comportemental et physiologique pour maîtriser une situation. Encore une fois, ce concept est proche de celui de syndrome comportemental. Cependant, il

semble mettre l'emphase sur les situations de stress. Une **stratégie comportementale d'adaptation** (**coping strategy**) est un ensemble cohérent de réponses (comportementales et physiologiques) à un stress, caractéristique d'un groupe d'individus (Carere *et al.*, 2003; Koolhass *et al.*, 1999). Koolhass *et al.* (1999) semblent considérer que la distribution des comportements est uniquement binomiale, basée sur les extrêmes présents dans chaque population. Les stratégies comportementales d'adaptation sont basées sur les travaux de Henry et Stephens (1977) qui suggèrent que deux types de réponses doivent être distingués (Koolhass *et al.*, 1999). Ainsi, les individus **proactifs** interagissent avec la source de stress de façon à modifier la situation, alors que les **réactifs** l'évitent (Tableau I.1). Le test d'enfouissement chez le rat (*Rattus norvegicus*) permet de visualiser concrètement la différence entre ces deux stratégies comportementales d'adaptation. Dans ce test, une électrode est introduite dans la cage de l'animal, qui reçoit des petits chocs électriques lors de son contact. En réponse à cette intrusion, un individu proactif enfouit l'électrode sous les copeaux de sa cage alors qu'un individu réactif évite l'électrode en s'éloignant et en s'immobilisant vers le fond de la cage (Koolhass *et al.*, 1999). Auparavant, les termes « actif » et « passif » (Benus *et al.*, 1990) étaient utilisés à la place de « proactif» et de « réactif», mais ces termes ne décrivaient pas la différence fondamentale entre les deux stratégies et laissait supposer que les individus passifs n'expriment aucune réponse (en fait l'évitement est aussi une réponse). Par ailleurs, les réactifs sont flexibles et réagissent aux conditions environnementales en permanence. A l'inverse, les proactifs semblent essentiellement guidés par des mécanismes internes et peu sensibles aux stimuli environnementaux (Carere *et al.*, 2003). Ils développent facilement des routines (Benus *et al.*, 1990) et ont donc un type de comportement plus rigide (Carere *et al.*, 2003; Koolhass *et al.*, 1999).

Tableau I.1 Différences comportementales et hormonales entre des individus proactifs et réactifs (tiré de Carere, 2003; Koolhass *et al.*, 1999)

Variables mesurées	Stratégie comportementale d'adaptation	
	Proactif	Réactif
Latence d'attaque d'un intrus	Faible	Forte
Formation de routine	Forte	Faible
Latence d'approche d'un nouvel objet	Faible	Forte
Vitesse d'exploration dans un environnement nouveau	Forte	Faible
Prise de risque	Forte	Faible
Taux de testostérone en réponse au stress du à une manipulation	Faible	Fort

L'approche expérimentale a été privilégiée par les études biologiques et écologiques sur le tempérament; le tempérament étant mis en évidence en plaçant l'animal dans une situation stressante (Réale *et al.*, unpublished). La plupart des tests de tempérament, comme le test de l'arène (open-field) ou celui de la planche à trou (hole-board), considèrent la nouveauté comme la composante environnementale à l'origine du stress. Le test de l'open-field consiste à placer l'animal dans une enceinte fermée et à mesurer certains indicateurs de l'émotivité (urination et défécation), de la néophobie (temps de latence pour entrer), et ou de l'activité et de l'exploration combinées (distance parcourue (Archer, 1973) et nombre de virages (Bronikowski *et al.*, 2001)). L'interprétation de la distance parcourue comme un indice d'exploration dans l'open-field est plutôt controversée car la locomotion dépend de l'activité de l'animal (File & Wardill, 1975; Walsh & Cummins, 1976). Le test du hole-board a ainsi été développé pour obtenir une mesure d'exploration indépendante de l'activité (File & Wardill, 1975). Ce test est une variante de l'open-field équipée d'une planche à trou sur le fond. La fréquence et le temps passé par l'animal la tête dans un trou est une bonne mesure de l'exploration (File & Wardill, 1975). Ce test est largement utilisé dans les recherches portant sur la personnalité ou en neurobiologie.

Pour mesurer la variabilité entre les individus et la consistance individuelle des comportements dans une population, les animaux doivent être placés de manières répétées, au moins deux fois, dans une même situation expérimentale (Hayes & Jenkins, 1997; Henderson, 1990). Lorsque le même test, basé sur la nouveauté, est répété de nombreuses fois, la nouveauté du test ne persiste peut-être pas. De plus, les individus peuvent varier dans leur habituation au nouvel environnement. Jusqu'à présent, peu d'études se sont intéressées à la variation interindividuelle de l'habituation aux conditions expérimentales (Sih *et al.*, 2004) et à ces effets sur les mesures de tempérament ou de personnalité. Les méthodes classiques pour estimer la répétabilité (voir Lessels & Boag, 1987) ne considèrent pas la variation interindividuelle dans l'habituation et peuvent biaiser l'estimation de la consistance

individuelle. De plus, l'absence de variation interindividuelle dans l'habituation indiquerait l'existence d'une consistance individuelle inter-environnementale (behavioural carryover) avec la diminution de nouveauté. Finalement, les études sur l'habituation peuvent apporter des informations importantes sur l'écologie d'une espèce, notamment sur la façon avec laquelle les individus s'habituent à différentes situations. Par exemple, il est possible de tester si l'habituation peut être une facette de la personnalité, si l'habituation varie en fonction du risque de la situation ou si elle varie entre les sexes ou les classes d'âges. Nous proposons d'utiliser une méthode statistique basée sur l'utilisation du modèle linéaire mixte pour tester les différences entre les individus dans la réponse à la nouveauté, la variabilité interindividuelle dans l'habituation et l'existence de corrélation entre la réaction à la nouveauté et l'habituation (Steele & Hogg, 2003)

Les études sur la variabilité interindividuelle ont été développées majoritairement sur des animaux captifs, sélectionnés artificiellement (rongeur Archer, 1973; oiseau, Carere *et al.*, 2003; File, 2001; cochon, Hessing *et al.*, 1994; Koolhass *et al.*, 1999) ou sur des animaux sauvages testés en laboratoire (oiseau, Dingemanse *et al.*, 2003; poisson, Pilastro, Benetton & Bisazza, 2003). À quelques exceptions, les animaux ont été testés directement sur le terrain (Armitage, 1986; Coleman & Wilson, 1998; Reale *et al.*, 2000; Wilson *et al.*, 1993). Cependant, les mesures de tempérament d'animaux sauvages directement sur le terrain ont de nombreux avantages par rapport aux mesures en laboratoire. Le premier avantage, vraisemblablement le plus important pour l'étude de l'écologie du tempérament, est de limiter les effets secondaires potentiels dus au transport, à la mise en captivité et au relâchement des animaux dans leur habitat d'origine après les expériences en laboratoire. L'ensemble de ces manipulations peuvent affecter l'écologie et le comportement des animaux étudiés (ce qui n'est pas souhaitable). Par exemple, un animal territorial gardé captif pendant 24 à 48 heures peut perdre son territoire ou avoir à affronter un compétiteur. Les animaux sociaux peuvent de la même manière

voir leur rang de dominance affecté par ces longues manipulations en laboratoire. Ces effets peuvent entraîner des coûts potentiels sur la survie ou l'aptitude des individus. Les tests en laboratoire, sur des animaux sauvages récemment capturés, peuvent aussi avoir un impact important sur la réponse au stress, en créant des conditions trop stressantes pour les individus et en produisant des mesures peu appropriées du tempérament de l'animal dans une situation naturelle. Finalement, les mesures de tempérament sur le terrain permettent de tester directement des hypothèses reliées au rôle écologique du tempérament. Par exemple, le même test réalisé dans deux types de micro-habitats différents dans leurs risques de prédation peut permettre de mesurer le stress induit par chaque habitat spécifique pour chaque individu. Pour cette maîtrise, nous avons utilisé un test de hole-board et un test du sac de capture réalisés de manière répétée sur plusieurs individus dans une population naturelle de tamias rayés (*Tamias striatus*). Les tamias sont des rongeurs diurnes habitant les forêts décidues de l'est de l'Amérique du nord (Snyder, 1982). Ils sont faciles à capturer et à observer, ce qui facilite les expériences sur le terrain. Nous avons développé des tests de tempéraments de manière à les réaliser juste après la capture d'un tamia et à limiter le temps d'expérience. Nous avons aussi testé l'effet possible du risque de prédation sur le comportement en réalisant, pour le même individu, le test du hole-board dans deux types d'habitats (milieu ouvert et sous canopée).

Dans la réserve naturelle Gault (Canada), depuis de nombreuses années, le tamia rayé est fortement en contact avec le public et est souvent nourris par les touristes. L'idée selon laquelle la perturbation humaine induit un stress chez les animaux est maintenant largement acceptée (Carney & Sydeman, 1999; Dyck & Baydack, 2004; Fowler, 1999). En outre, certaines études ont montré des changements physiologiques et comportementaux chez les animaux sauvages exposés à une perturbation humaine (Fowler, 1999; Romero & Wikelski, 2002; Walker, Boersma & Wingfield, 2005). Ces études ont été publiées en même temps que grandit l'intérêt pour une approche basée au niveau de l'individu (Mcdougall *et al.*, 2006).

Cependant, très peu d'études se sont intéressées à comprendre comment, selon leur tempérament, les individus peuvent montrer des réponses différentes à une perturbation humaine (Mcdougall *et al.*, 2006). Les différences comportementales de tempérament sont fortement associées avec une réponse hormonale spécifique (Buss *et al.*, 1987). Par exemple, en réponse à un stress, les animaux émotifs, timides et peu explorateurs (animaux réactifs *sensu* Koolhass *et al.*, 1999) montrent habituellement un plus fort pic de cortisol que les individus actifs, non dociles et très explorateurs (individus proactifs *sensu* Koolhass *et al.*, 1999). De plus, la variation dans l'exploration entre les individus a un effet sur leur potentiel à se disperser (Dingemanse *et al.*, 2003; Fraser *et al.*, 2001). Ainsi, les individus d'une population pourraient, selon leur tempérament, varier dans leur potentiel à occuper des zones plus ou moins perturbées (ex: les zones perturbés pourraient être favorisées par les animaux explorateurs, proactifs). En conséquence, les différences hormonales mesurées sur des animaux occupant des zones perturbées ou non perturbées pourraient ne pas être seulement un effet direct de la perturbation humaine, mais aussi refléter une distribution non aléatoire des individus avec des tempéraments différents (et donc des réponses hormonales différentes).

Dans un premier chapitre, nous proposons une méthode dérivée du test du hole-board pour étudier le tempérament chez des animaux sauvages directement dans leur habitat naturel et l'approche statistique du modèle mixte qui permet d'estimer la variation interindividuelle de la réaction comportementale à une situation nouvelle et stressante et de l'habituation à des tests répétés (ex : diminution de la nouveauté). Nos objectifs étaient de tester si : 1) les tamias variaient dans leur réponse comportementale à une nouvelle situation (hole-board ou sac de capture); 2) les tamias s'habituaient à la situation expérimentale; 3) la réaction à la nouvelle situation et l'habituation variait avec le risque de la situation; 4) on pouvait mettre en évidence une consistance individuelle inter-environnementale (behavioural carryover *sensu* Sih *et al.*, 2004) dans la réponse comportementale des tamias au test. Finalement, à partir

du modèle linéaire mixte, nous pouvons obtenir des valeurs individuelles de comportement et d'habituation qui représentent des valeurs de profil comportemental individuel. En utilisant ces valeurs, nous nous sommes intéressés aux corrélations phénotypiques entre ces comportements.

Dans un deuxième chapitre, nous avons examiné les relations existant entre les profils de comportement obtenus (traits de tempérament) et la perturbation humaine. Ensuite, nous avons observé l'effet du tempérament et de la perturbation humaine sur la réponse hormonale au stress (concentration de cortisol) à deux échelles temporelles différentes : 1) en analysant l'accumulation de cortisol, dans des poils récoltés sur chaque tamia, comme un indice du stress chronique, et 2) en analysant la concentration fécale de cortisol illustrant la réponse à court terme aux stress environnementaux. Nous discutons finalement les implications de ces phénomènes pour les études sur l'impact de la perturbation humaine sur la vie sauvage.

CHAPITRE I

TEMPERAMENT AND HABITUATION TO NOVELTY: A FIELD STUDY IN A WILD POPULATION OF EASTERN CHIPMUNKS (*TAMIA STRIATUS*)

ABSTRACT

Studies of animal temperament have mostly been performed by running experimental tests where an animal is placed in a novel, stressful situation, and its behavioural-hormonal reactions recorded. One important step in the study of temperament is to show repeatability of traits. This can only be accomplished by doing several replicates of the same test on the same individuals. However, if animals become habituated to the test, their behavioural reaction may be affected. As a result, the measure of reaction to novelty and its repeatability can be biased by potential interindividual differences in the rate of habituation and micro-environmental conditions during the first test. In this study we used an approach that permitted us to estimate individual variation in the behavioural reaction to novelty and in the habituation to experimental tests. We repeatedly recorded the behaviour of 24 individually marked Eastern chipmunks (*Tamias striatus*) in a hole-board and a handling bag test. Three main temperament components emerged from a principal component analysis of hole-board behaviours: exploration/activity, stress reaction and emotionality. One docility index could be estimated from the reaction of individuals to the handling bag test. We then estimated reaction to novelty and rate of habituation for each individual using a linear mixed-model. Our results showed high intra-individual consistency for the four temperament traits. Furthermore we found a strong behavioural carryover for habituation to novelty. The absence of phenotypic variation in habituation could have consequences for the evolutionary potential of habituation to novelty in this chipmunk population.

Keywords: hole-board, temperament trait, habituation, behavioural syndrome, exploration, docility, chipmunks.

INTRODUCTION

Recently, a growing number of studies in animal behaviour have focused on the individual variation in behaviours in wild populations (Carere *et al.*, 2005; Dingemanse *et al.*, 2003; Hayes & Jenkins, 1997; Reale *et al.*, 2000; Sih, Bell & Johnson, 2004; Wilson, 1998), and have explored the evolutionary and ecological implications of that variation (Carere *et al.*, 2005; Dingemanse *et al.*, 2003; Reale & Festa-Bianchet, 2003). Individual variation in behaviour has been called coping style (Koolhass *et al.*, 1999), temperament (Buss & Greiling, 1999), personalities (Gosling, 2001), or behavioural syndromes (Sih *et al.*, 2004) depending on the author, and these terms are sometimes considered synonymous (Reale *et al.*, unpublished). For example temperament is defined as the individual consistency in behaviours over time and across situations (Buss & Greiling, 1999), and a behavioural syndrome represents an association (*i.e.* a genetic correlation) between two behaviour traits or between two expressions of the same trait across different contexts and situations (Clark & Ehlinger, 1987; Sih, Bell & Johnson, 2004; Sih *et al.*, 2004).

To measure inter-individual variation and behavioural individual consistency in a population, animals should be placed repeatedly (at least twice) in the same experimental situation (Hayes & Jenkins, 1997; Henderson, 1990). Most tests of temperament consider novelty as the environmental component at the origin of the stress. However, novelty may not persist when the same test is replicated several times and individuals may differ in their habituation to novel conditions. Yet no studies have investigated inter-individual variation in habituation to an experimental situation (Sih *et al.*, 2004) or its effect on the measurement of temperament or personality. Classical methods to estimate repeatability (see Lessels & Boag, 1987)

do not consider inter-individual variation in habituation and may bias the estimate of behavioural consistency. For instance, repeatability of a temperament trait may be underestimated if individuals with a high score at the first test habituate faster than individuals with a low score. Furthermore, an absence of inter-individual variation in habituation would indicate the existence of a behavioural carryover (Sih, Bell & Johnson, 2004) with the decline in novelty. Studies of habituation and temperament could also provide insights on the behavioural ecology of habituation. For example habituation could be considered a facet of personality, and play an important role in how an animal cope with habitat heterogeneity, predation risk, or human disturbance.

In this paper, we propose to use a mixed-model approach to estimate inter-individual variation in both the behavioural reaction to a new stressful situation and in habituation to novelty (*i.e.* repeated tests). We performed repeated hole-board tests and handling bag tests on wild Eastern chipmunks (*Tamias striatus*), in two different environmental situations with different levels of predation risk (*i.e.* low: under canopy; high: open area). Chipmunks are diurnal rodents living in the hard-wood forest of Eastern North America (Snyder, 1982). They are easy to capture and to observe, which facilitates field experiments.

Our objectives were to test if: 1) chipmunks differed in their behavioural reaction to a novel situation (*i.e.* the hole-board-test or the handling bag test); 2) chipmunks habituated to the experimental test; 3) reaction to a novel situation and habituation differed with situation risk; and 4) there was a behavioural carryover in chipmunks' behavioural reaction to the test. Finally, from the mixed linear model we predicted individual values for behaviours and habituation that could be used as individual values of behavioural profiles. Using these values we examined phenotypic correlations between those behaviours.

METHODS

Study Area and Subjects

Field work was conducted in a deciduous forest in the public area of the McGill University field station at Mont Saint Hilaire, Quebec, Canada, from May 2 to September 22 2004. The area is covered by a network of public trails. Access is restricted to the trails, and thus the forest is relatively undisturbed by people. Because of frequent contacts with humans in the area, chipmunks are habituated to their presence and are often fed by the public. Chipmunks are solitary, territorial, central place foraging rodents (Elliott, 1978). They are active above ground from April to October. During this period, they intensively build up food reserves in their burrow that they use for hibernation in winter.

Trappings and Markings

Chipmunks were live-trapped with Sherman traps baited with sunflower seeds. We identified the burrows of 24 chipmunks previously trapped, sexed, ear-tagged (National Band and Tag Co., New-Port, Kentucky) and fur-clipped for identification at a distance (Elliott, 1978; Giraldeau *et al.*, 1994). Chipmunks were weighed to the nearest gram, using a spring balance (Pesola). Some chipmunks were already marked from other studies prior to 2004 (studies have been conducted in the area since 1984 with some interruptions). Due to a lack of long-term information, chipmunks could not be aged precisely. We therefore considered two age categories: individuals captured prior to 2004, and individuals captured for the first time in 2004. For each chipmunk distance between the burrow and the closest public trail was

measured. We differentiated chipmunks with a burrow located more than 5m from a trail from those whose burrow was located within 5 m of a trail.

Behavioural Observations

Behavioural observation of animals in the wild alone hardly permits measurements of between individual variation, and an experimental and ethological approach is thus recommended (Reale *et al.*, unpublished). Field temperament tests were developed to be run just after trapping so that we limited the time between the capture of an individual and its release after the experiment.

Handling Bag Test

Each time a chipmunk was captured for routine measurements it was tested in the handling bag test. The handler held the handling bag containing the chipmunk, and measured the proportion of time the chipmunk spent immobile during one minute. This test was performed to measure the behavioural response to human presence and manipulation. In a farm mink, reaction towards the presence of humans, static or walking, is used as a measure of docility of an individual (Korhonen, Jauhiainen & Rekila, 2002). Measuring static time in a handling bag might correspond to a measure of docility for chipmunks. We collected data for an average 6 replicated tests (range 2-10) on 29 chipmunks.

Hole-board Test

The open-field test has been largely used to measure individual variations in behaviour. The test consists of measuring the behavioural reaction of an animal in a novel environment from which escape is prevented by a surrounding wall (Walsh & Cummins, 1976). Open-field measures provide information on animal activity,

exploration and emotionality (Archer, 1973; Walsh & Cummins, 1976). The use of the animal's locomotion in an open-field as a measure of exploration is controversial because the animal's locomotion depends on its activity (File & Wardill, 1975; Walsh & Cummins, 1976). The hole-board test, a derivate of the open-field apparatus with holes in the floor, permits to use head-dipping (*i.e.* the animal puts his head in a hole) frequency and proportion as measures of exploration independent of activity (File & Wardill, 1975).

The hole-board was constructed from a white rectangular plastic box (80 x 40 x 40 cm) with a plexiglass top and an entrance lock on one of the longer sides (Fig 1.1). There were four holes in the floor equally distant from the entrance lock, each 5 cm in diameter and 4 cm deep. Two holes were in the center and two in the corners of the box, respectively at 15 and 5 cm from the nearest wall.

To limit the effects of burrow distance and trapping stress, chipmunks were trapped between 2 and 5 meters from their burrow and spent an average of 15min (range: 10-20min) in the trap before a test. Animals were not manipulated prior to the hole-board test; they were directly placed in the entrance lock for one minute. We then opened the hole-board door and let the animal enter the arena. After a 10 min period, a chipmunk that had not entered the hole-board was gently pushed in the arena by slowly moving the external door toward the arena. Once the chipmunk was in the arena we closed the hole-board door. Chipmunk behaviours were then recorded for 5min with a camcorder (Canon ZR 80). The hole-board was cleaned with water and a cloth between tests.

Video images were later analysed using The Observer 5.0 software (Noldus, Wageningen, The Netherlands). We measured the eight following behaviour variables:

- Latency to leave entrance lock (in seconds; with a maximum of 600s when animals pushed in the hole-board after 10min).

- Scanning: proportion of time spent moving head.

- Grooming: proportion of time spent grooming.

- Locomotion: proportion of time spent walking or running.

- Rearing: proportion of time spent rising up on hind legs.

- Climbing: proportion of time spent hanging on top of walls.

- Head-dipping: proportion of time spent looking down in a hole. A head-dip was scored when both eyes disappeared into the hole (File & Wardill, 1975; Semenova *et al.*, 2001).

- Defecation and urination: sum of number of defecation and urination events during the test (Archer, 1973; Wilson *et al.*, 1976).

Hole-board tests were run in two different environmental situations: 1) under forest cover, and 2) in an open area. These two types of micro-habitats are assumed to differ in terms of predation risk. When attacked by a predator, chipmunks seek cover in holes, under logs, or occasionally climb trees (Bonenfant & Kramer, 1996; Clarke *et al.*, 1993). Moving into open areas is therefore likely to increase predation risk because of increased distance from a suitable refuge. Furthermore, chipmunks differ in their locomotion when moving under the forest cover or in an open area, spending more time pausing when moving away from forest cover (Mcadam & Kramer, 1998). A forested area was thus considered a low predation risk situation compared to an open area. Between one and three tests were performed per environment type and per chipmunk, with a minimum of 10 days between two successive tests. Date, time of the day and order of the test were also noted for each test. We collected data for 18 chipmunks with two to three tests in each of the two environmental situations and for another sample of six chipmunks with at least two tests, regardless of the environmental situation.

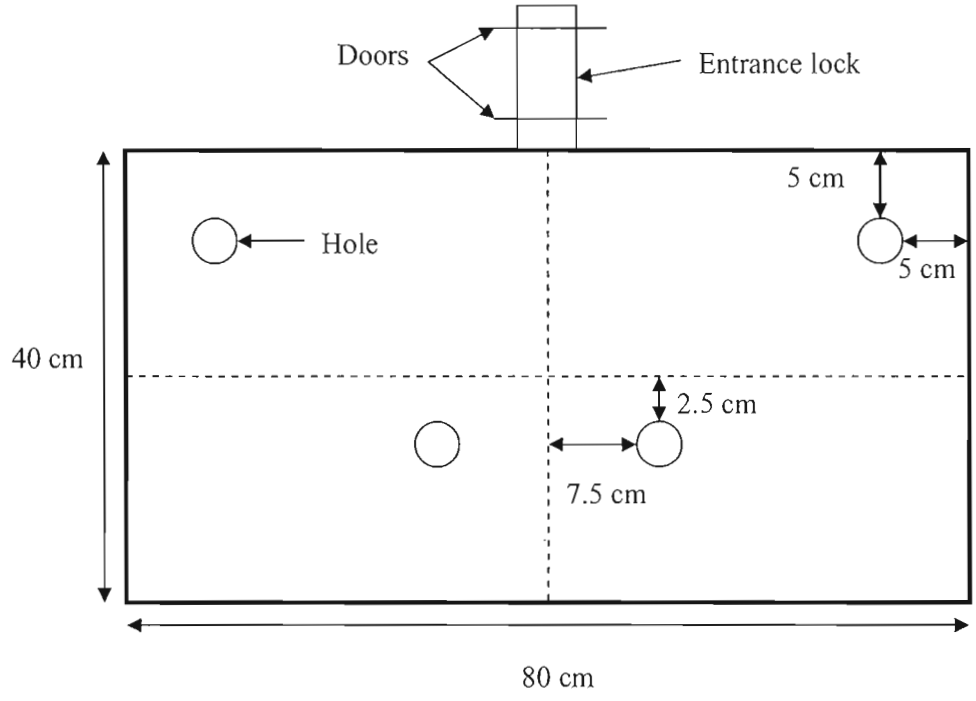

Figure 1.1 Top view of a modified hole-board.

Statistical Analyses

Principal Component Analysis

We used a principal component analysis on the correlation matrix of behaviour variables in the hole-board to illustrate the relationship between behaviour variables, and to reduce the number of behaviour variables prior to additional statistical analyses. The construction of principal components does not require that variables show a multivariate normal distribution (Timm, 2002), and thus untransformed variables were used. The Kaiser-Guttman criterion was used to select the number of principal components to retain (Kaiser, 1991). Each retained principal component was then used as a composite behaviour variable.

Linear Mixed-Model

Prior to analyses, static time in handling bag was square-root transformed to obtain normality. Restricted maximum likelihood (REML) linear mixed model analyses were run on the time spent immobile in the handling bag and on the composite behaviour variables, using date, hour, environment type (*i.e.* forested or open area), trial order, sex, capture year, weight and distance to the path as fixed effects. Chipmunk identity was included in the model as a random effect. Comparison of the two models with and without chipmunk ID, allowed us to test for the significance of variance in a behavioural index caused by individual differences (Pinheiro & Bates, 2000). To test for the significance of between-individual variation in phenotypic plasticity, we compared models with and without the interaction between chipmunk ID and date, hour, environmental situation, and trial order, respectively. The additional explanatory power of adding one random effect to a model, provided the fixed-effect structure remained the same, can be measured using a log-likelihood ratio test (LRT) (Pinheiro & Bates, 2000). We calculated LR as 2

[log-likelihood of model B – log-likelihood of model A], where model B is a more general model than model A (*i.e.* model A has fewer random effects). The resulting log-likelihood test statistic distribution approximates a χ^2 distribution with k_B-k_A degrees of freedom, where k_i is the number of parameters to be estimated in model *i* (Pinheiro & Bates, 2000). In the mixed-effect model including chipmunk ID as a random effect, the fixed-effect estimate of an environmental covariate provides an estimate of the overall plastic response of individuals to that variable. A significant fixed-effect indicates that on average individuals are plastic (see fig. 1.2c or 1.2d). A significant interaction between chipmunk ID and one environmental variable indicates some between-individual variation in plastic response of individuals to the variable (see Fig. 1.2a or 1.2d). Interaction between chipmunk ID and an individual fix trait as sex could not be tested. Because observations were made over the same year, age and distance to nearest path were fixed within an individual; therefore interaction with chipmunk ID could not have been tested.

Correlation between temperament variables

Best linear unbiased predictors (BLUPs) for random effects (*i.e.* chipmunk ID) within the mixed models were used as estimates of individual behaviour profiles. BLUP method provides estimates of random effects independent of other terms within a model, standardised to a mean of zero (Kruuk, 2004). They also have the advantage of being less sensitive to extreme values within data than separate regression estimates (Pinheiro & Bates, 2000). Therefore they provide more appropriate estimates of the behaviour profile of an individual than the mean of all the measures for that individual. The model with the significant effects only was used to calculate the BLUPs. We estimated phenotypic correlations between hole-board behaviour profiles and handling bag profiles. Statistical analyses were performed using R 2.1.0 (Ihaka & Gentleman, 1996).

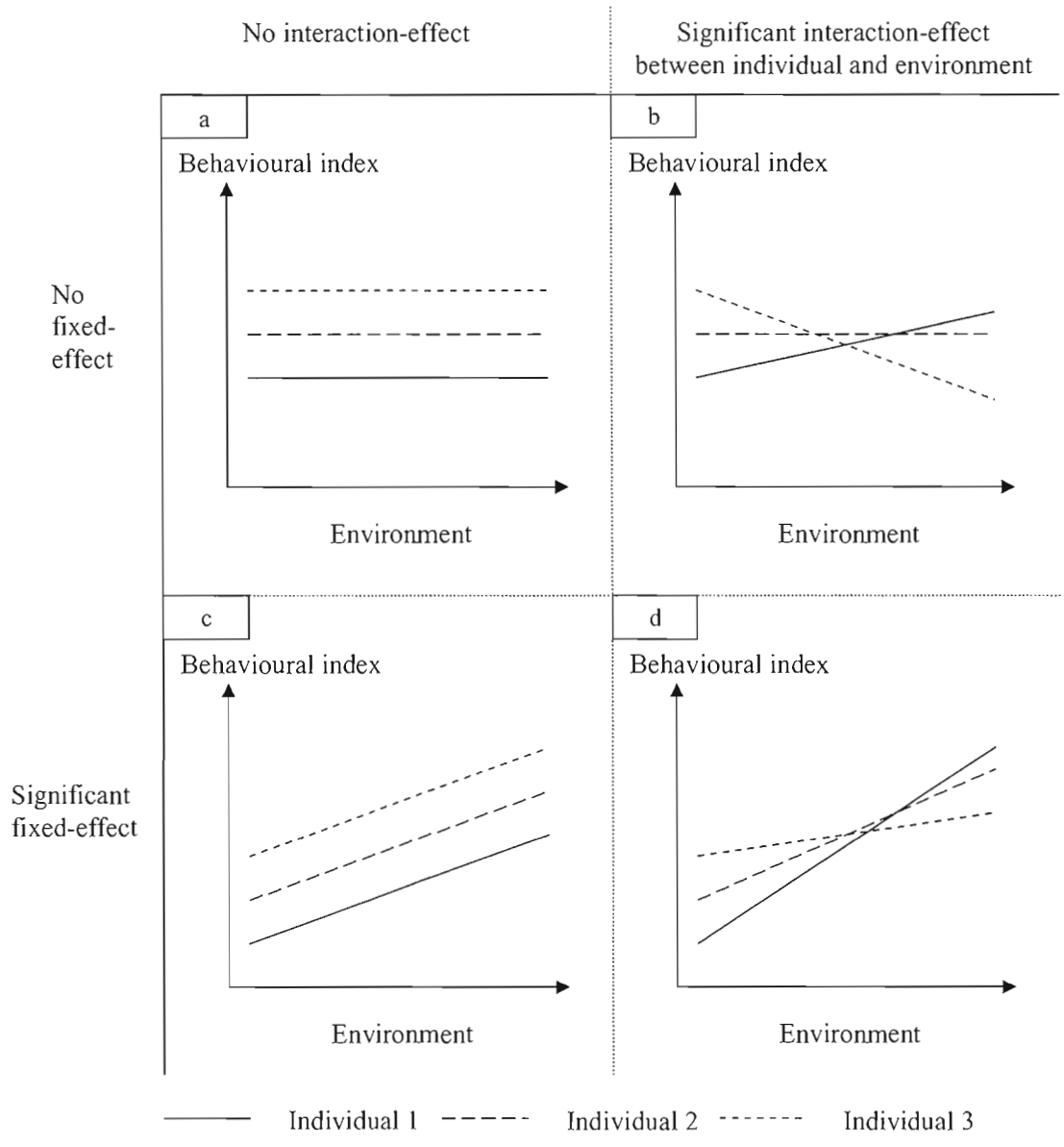

Figure 1.2 Behavioural index trends across an environmental gradient for three hypothetic individuals, illustrating the main patterns of plasticity (adapted from Pigliucci, 2001): (a) Variation in elevation (trait means) but no average plasticity or variation in plasticity; (b) No overall plastic response but variation in plasticity; (c) Average plastic response without variation in plasticity; (d) Average plastic response with variation in plasticity. Figures 1.2a and 1.2c illustrate the potential for a behavioural syndrome.

RESULTS

Analyses were first conducted on 18 chipmunks with data from at least two tests in each of the two environmental situations. Environmental situation was included as a fixed effect in the mixed-model. Neither environmental situation nor the interactions between chipmunk ID and environmental situation had significant effects on the three behavioural components of the hole-board test ($P > 0.44$ for the two effects on the three components). We thus removed environmental situation from the following analyses and ran analyses with a database of 24 chipmunks for which we had data on at least two tests, regardless of the environmental situation.

Three variables, explaining 66% of the total variance, were retained in the principal component analysis (Table 1.1). The first component represented activity and exploration, with locomotion, climbing, rearing, and head-dipping mainly contributing to the left hand side of axis 1 (Table 1.1). The second component was bipolar, opposing scanning and grooming behaviour with urination-defecation index (Table 1.1). The third component was dominated by latency to enter and the urination-defecation index (Table 1.1).

Chipmunk identity explained a significant proportion of the variance in the three principal components (PC1: 42.99%; PC2: 23.39%; PC3: 34.39%) and in static time (23.83%; Table 1.2). None of the interactions between chipmunk ID and date, hour or trial order significantly improved the model fit for any of the behavioural variables tested (Table 1.2), indicating that individual behavioural responses to change in these environmental variables were not significantly different (similar to patterns in Fig. 1.2a and 1.2c).

Because of the absence of significant interactions between ID and environmental variables, chipmunk identity alone was included in the next model that tested the effects of state or of environmental variables on behavioural variables (Table 1.3). Therefore we assumed patterns similar to Fig. 1.2a or 1.2c for these models. Chipmunks decreased significantly their activity/exploration (*i.e.* PC1) with the number of trials in the hole-board (Table 1.3 and Fig 1.3a). Activity/exploration decreased significantly with distance between burrow entry and the nearest trail (Table 1.3 and Fig 1.3b) and with age (Table 1.3 and Fig 1.3c). Animals first captured before 2004 urinated and defecated more, and groomed and scanned less (*i.e.* PC2) than animals captured for the first time in 2004 (Fig 1.3d). Behavioural stress response (*i.e.* PC3) to the hole-board decreased with the number of trials (Fig 1.3e). In the handling bag, chipmunks' static time decreased significantly with repeated tests and with body weight (Table 1.3). No significant correlations were found between BLUP values of the three principal components of the hole-board test and the BLUP value of static time in the handling bag ($r < 0.25$ and $P > 0.42$ for all the correlations).

Table 1.1 Summary of principal-component analysis of the hole-board test data for chipmunks at Mont-St-Hilaire. Coefficient larger than 0.4 in absolute value are in bold. Using Kaiser-Guttman criterion, components not retained for further analysis are in grey.

	Component							
Variables	1	2	3	4	5	6	7	8
Locomotion	**0.54**	-0.13	0.10	-0.12	-0.06	-0.07	-0.08	0.80
Rearing	**0.45**	0.05	0.13	0.39	0.07	-0.18	-0.67	-0.33
Climbing	**0.43**	0.10	-0.03	-0.17	0.73	-0.13	0.41	-0.19
Head-dipping	**0.43**	0.13	0.11	0.31	-0.56	0.01	0.56	-0.22
Scanning	0.19	**-0.55**	0.34	-0.57	-0.16	0.21	-0.07	-0.35
Grooming	-0.10	**-0.61**	0.20	0.60	0.28	0.29	0.17	0.11
Urination and defecation	-0.04	**0.52**	**0.61**	-0.01	0.12	0.56	-0.05	0.08
Latency to enter	-0.27	-0.02	**0.64**	-0.01	0.01	-0.70	0.11	0.05
Standard deviance	1.67	1.12	1.09	0.95	0.79	0.76	0.66	0.42
% of total variance	0.35	0.15	0.14	0.11	0.07	0.07	0.05	0.02
Cumulative proportion of total variance	0.35	0.51	0.66	0.77	0.84	0.92	0.97	1.00

Table 1.2 Explanatory power and significance of different random effects in linear mixed-models of behavioural variables (*i.e.* the first three components of the PCA on the hole-board test data and on static time in the handling bag test) for chipmunks at Mont St-Hilaire. Ticks indicate random effects fitted in respective models. Significant differences between models, based on log-likelihood tests, are in bold. Analysis of variance component of model indicated that Identity counted for 42.99%, 23.39%, 34.39% and 23.83% of the variance of the variables PC 1, PC 2, PC 3 and static time respectively.

	ID	ID * D.	ID * H.	ID * O.	Model	K	Loglik.	Test	LRT	DF	*P*
PC 1 (Activity / Exploration)					1	9	-159.26				
	O				2	10	-151.56	1 vs 2	15.40	1	**<0.001**
	O	O			3	12	-151.55	2 vs 3	<0.01	2	0.99
	O		O		4	12	-151.55	2 vs 4	<0.01	2	0.99
	O			O	5	12	-151.55	2 vs 5	<0.01	2	0.99
PC 2 (Reaction to stress)					1	9	-138.78				
	O				2	10	-136.53	1 vs 2	4.50	1	**0.033**
	O	O			3	12	-135.67	2 vs 3	1.73	2	0.42
	O		O		4	12	-135.88	2 vs 4	1.31	2	0.52
	O			O	5	12	-136.17	2 vs 4	0.73	2	0.69
PC 3 (Emotionality)					1	9	-141.26				
	O				2	10	-135.98	1 vs 2	10.56	1	**<0.001**
	O	O			3	12	-135.95	2 vs 3	0.04	2	0.97
	O		O		4	12	-135.51	2 vs 4	0.92	2	0.63
	O			O	5	12	-135.83	2 vs 5	0.30	2	0.86
Static time in handling bag					1	9	-276.53				
	O				2	10	-271.59	1 vs 2	9.86	1	**<0.001**
	O	O			3	12	-271.59	2 vs 3	<0.01	2	0.99
	O		O		4	12	-271.59	2 vs 4	<0.01	2	0.99
	O			O	5	12	-271.59	2 vs 5	<0.01	2	0.99

D.: date; H.: hour of day; O.: trial order

Table 1.3 Estimates of fixed effects produced by a linear mixed-model of behavioural indexes with chipmunk ID as random effect (for random effects see Table 1.2).

	PC 1 (Activity/Exploration)			PC 2 (Reaction to stress)			PC 3 (Emotionality)			Static time		
	Coefficient	Test statistic	*P*	Coefficient	Test statistic	*P*	Coefficient	Test statistic	*P*	Coefficient	Test statistic	*P*
(Intercept)	3.10 ± 1.02	$F_{1, 62} = 0.04$	0.83	1.60 ± 0.84	$F_{1, 62} = 0.00$	0.99	-0.32 ± 0.84	$F_{1, 62} = 0.02$	0.89	5.18 ± 0.92	$F_{1, 122} = 535$	**<0.001**
Date	0.00 ± 0.01	$F_{1, 62} = 0.19$	0.67	0.00 ± 0.01	$F_{1, 62} = 0.36$	0.55	0.01 ± 0.01	$F_{1, 62} = 0.61$	0.44	0.00 ± 0.01	$F_{1, 122} = 1.01$	0.31
Time	-0.00 ± 0.06	$F_{1, 62} = 1.18$	0.28	-0.08 ± 0.05	$F_{1, 62} = 1.69$	0.20	0.02 ± 0.05	$F_{1, 62} = 0.16$	0.69	-0.04 ± 0.06	$F_{1, 122} = 1.29$	0.25
Order	-0.78 ± 0.24	$F_{1, 62} = 45.2$	**<0.001**	0.12 ± 0.20	$F_{1, 62} = 0.03$	0.86	-0.44 ± 0.20	$F_{1, 62} = 5.27$	**0.025**	-0.14 ± 0.06	$F_{1, 122} = 10.1$	**0.002**
Weight	0.05 ± 0.05	$F_{1, 19} = 0.95$	0.34	0.00 ± 0.03	$F_{1, 19} = 0.00$	0.97	0.05 ± 0.03	$F_{1, 19} = 2.21$	0.15	-0.07 ± 0.03	$F_{1, 24} = 5.11$	**0.033**
Sex[1] (Male)	-0.39 ± 0.56	$F_{1, 19} = 0.11$	0.74	0.45 ± 0.38	$F_{1, 19} = 2.00$	0.18	0.20 ± 0.42	$F_{1, 19} = 0.28$	0.60	0.51 ± 0.40	$F_{1, 24} = 0.02$	0.88
First capture year[2] (< 2004)	-1.46 ± 0.49	$F_{1, 19} = 4.93$	**0.039**	-0.93 ± 0.33	$F_{1, 19} = 10.1$	**0.005**	-0.03 ± 0.37	$F_{1, 19} = 0.01$	0.92	-0.01 ± 0.39	$F_{1, 24} = 0.00$	0.98
Distance to trail[3] (>5m)	-1.19 ± 0.47	$F_{1, 19} = 5.84$	**0.026**	0.17 ± 0.32	$F_{1, 19} = 0.29$	0.59	-0.09 ± 0.35	$F_{1, 19} = 0.00$	0.96	-0.65 ± 0.36	$F_{1, 24} = 2.92$	0.10

[1] female was considered as the reference for sex in the model. [2] year 2004 was considered as the reference for first capture year; and [3] <5m was considered as the reference for distance between burrow and the nearest trail in the model.

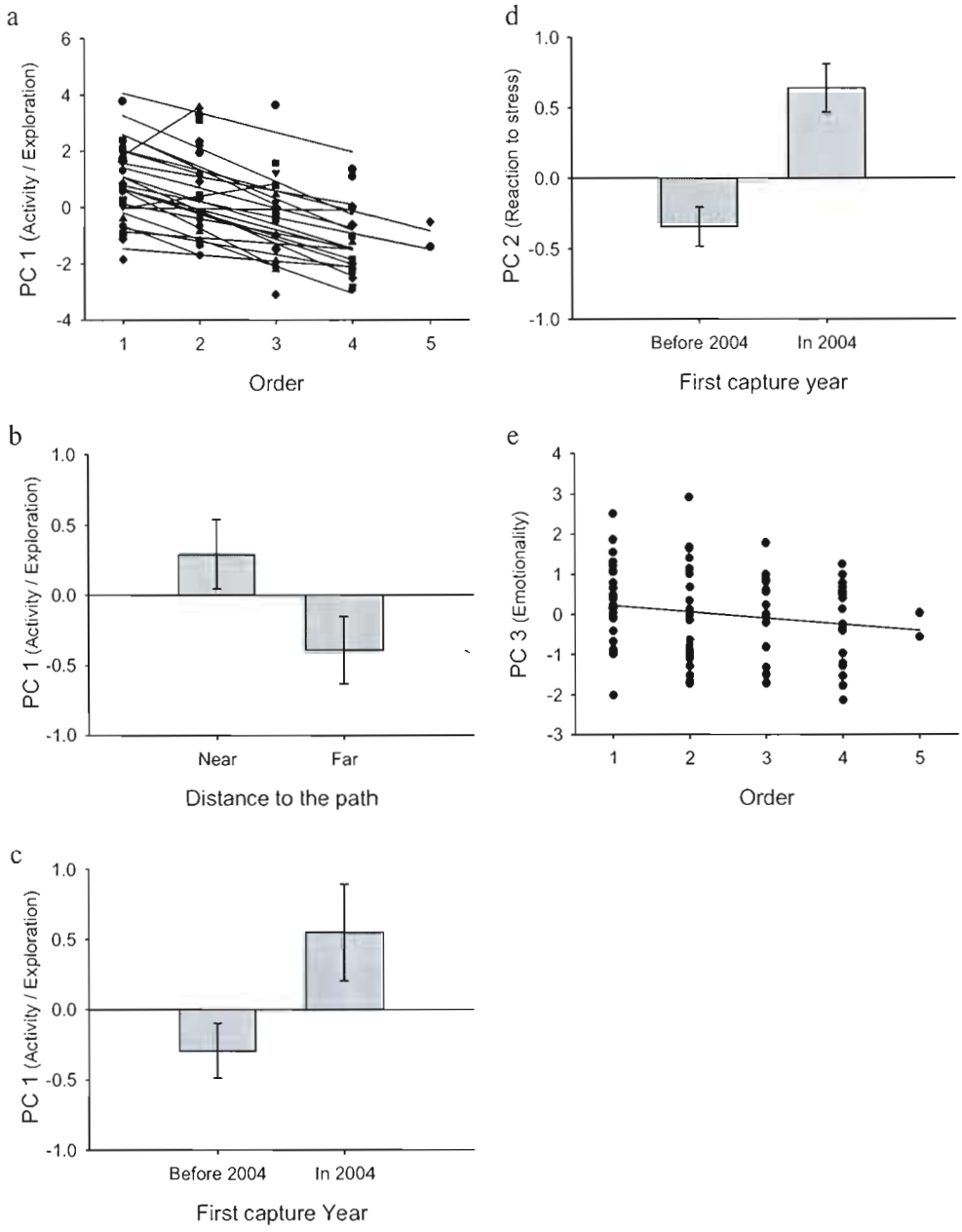

Figure 1.3 Significant effects on behavioural indices obtained from the PCA analysis of hole-board data in the chipmunk population of Mont-St-Hilaire. Effects of a) trial order for each individual; b) first year capture; c) distance between the burrow entry and the closest trail on PC 1; d) first year capture on PC 2; and e) trial order on PC 3. PC 1, PC 2 and PC 3 could be interpreted as Activity/Exploration, reaction to stress and emotionality respectively (see text for more details).

DISCUSSION

This is the first study on the inter-individual variation in behaviour to directly test, in the field, how individual animals vary in their habituation to novelty. Our results show that chipmunks habituated to the tests, by reducing their behaviours (PC 1 and PC 3) in the hole-board test and by increasing docility in the handling bag test. Speed of habituation, however, did not differ between individuals or situations. As a result, chipmunks showed constant ranking in temperament traits over time.

Interpretation of the behavioural variables in the hole-board test

From the principal component analysis of chipmunk behaviours in the hole-board we extracted three different behavioural components. Locomotion, rearing, climbing and head-dipping behaviours were all grouped within component 1. Locomotion, rearing and climbing reflected activity and exploration (File & Wardill, 1975). Head-dipping has been proposed as an exploration measure independent of activity (File & Wardill, 1975). File and Wardill (1975) proposed the hole-board test in response to the controversy regarding the inability of the open-field test to separate activity from exploration in small rodents. We used the hole-board to circumvent this problem. However, despite these experimental precautions, locomotion and head-dipping remained strongly associated, with highly active chipmunks also exploring holes for a higher proportion of time. From this result, we can conclude that activity and exploration in chipmunks are associated in a behavioural syndrome (see below).

The second component in the principal component analysis contrasted scanning, grooming and the urination-defecation index. All these behavioural variables have been identified as reactions to a stress (Daniels, Richter & Stein, 2004;

Kalueff & Tuohimaa, 2004a; Koya *et al.*, 2005). Rodents' grooming activity generally increases in two opposite situations: high and low stress (Kalueff & Tuohimaa, 2004b). A recent study by Kalueff and Tuohimaa (2005) on the differences between the grooming patterns of rodents in a comfort and in a stressful situation could help us interpret our result. They indicated that in a stressful situation grooming was characterised by frequent and rapid short bursts, while a general pattern of self-grooming with an uninterrupted cephalocaudal progression was normally observed in no-stress conditions. During the hole-board test, we observed frequent and short bouts of paw licking, and nose and face washing but almost never observed a complete cephalocaudal progression of grooming. We could therefore interpret grooming in the hole-board as a stress response to novelty. Defecation-urination was opposed to grooming and scanning on this second axis, suggesting antagonistic physiological pathways for the expression of these two stress responses. Similarly Kalueff and Tuohimaa (2004a) found an antagonism between grooming and urination-defecation in a stressful situation for two genetically different mice strains. To our knowledge, the reason for the opposition of grooming and urination-defecation are not known and further studies are needed to better understand this pattern.

Urine/defecation index was also associated with latency to enter the hole-board. These two behaviours have often been considered measures of emotionality, or sympathetic nervous system activity in response to different stress stimuli (*e.g.* novelty, predation) (Archer, 1973). Component 3 which grouped those 2 measures could thus be interpreted as an emotionality index.

Principal component analysis (PCA) is often used with open-field, hole-board or elevated plus-maze tests (Carola *et al.*, 2004; Kanari *et al.*, 2005; Kim *et al.*, 2002). Although it is difficult to compare our results with those from other studies due to variation between studies in definition and in number of variables integrated in the

PCA, a component grouping exploration measures and one grouping emotionality measures have emerged from all of these studies.

Individual differences in behaviours and habituation

Behaviours were significantly repeatable over the summer, with r ranging between 0.23 and 0.43 (see Table 1.2). Individual chipmunks showed consistency over time for the four behavioural variables measured, indicating variation in temperament in this population of wild rodents. Exploration and docility are assumed to play an important role in many ecological variables like habitat use, predation avoidance (Sih, Bell & Johnson, 2004; Sih *et al.*, 2004), dispersal or populations genetic structure (Dingemanse *et al.*, 2003). Furthermore, exploration in great tits (*Parus major*), and boldness in bighorn sheep (*Ovis Canadensis*) have been shown to affect several components of fitness (Dingemanse & Reale, 2005). Future studies should investigate how temperament affects the way in which an individual interacts with its environment and how this influences individual fitness. Measuring the consistency of a trait over time is important for ecological studies, because this consistency is the guaranty that the trait is really a single trait (Hayes & Jenkins, 1997), and that the variation observed at the phenotypic level is not only caused by micro-environmental effects at the moment of the measurement. It is especially important for the study of temperament, which assumes consistency of a trait over time within individuals (Reale *et al.*, unpublished).

With repeated tests, we observed both a decrease of activity/exploration (PC 1) and of emotionality (PC 3) in the hole-board and a decrease of docility (immobility) in the handling bag (Fig. 1.3). Decrease of activity/exploration could result from a decrease in novelty or from an increase in stress due to repetition of test. However, because this decrease is combined with a decrease in emotionality with trial order, we suggest these patterns reflect habituation to novelty rather than an increase

in the stress generated by the test. Our handling bag test results were similar to those on restrain stress measured with tonic immobility duration where tonic immobility decreases with habituation (Heiblum *et al.*, 1998). Habituation in a hole-board or an open-field is often considered as a measure of learning (Dukas, 1998; File, 2001) and could be interpreted in term of information processing (Elliott & Grunberg, 2005). Persistent high levels of activity/exploration level suggest that the animal is not acclimating to the novel environment or is not processing information efficiently. In contrast, increasingly lower levels of activity suggest faster acclimation and more efficient processing of environmental cues. Deficiencies in processing novel information may decrease learning rates and interfere with an organism's ability to adapt effectively to its environment (Elliott & Grunberg, 2005). Individual differences in activity/exploration and docility could results from inter-individual differences in information processing capabilities. However, with mixed models, we can consider habituation as a trait itself and test for its inter-individual variability. None of the individual by trial order interactions tested were significant, which means that chipmunks did not differ in their habituation to the hole-board or to the handling bag. Thus, we conclude that individual differences in temperament were not due to inter-individual differences in learning (Dukas, 1998) or information processing capabilities (Elliott & Grunberg, 2005). Furthermore, we can infer the presence of a behavioural carryover in chipmunks' activity/exploration, emotionality and docility with the decrease in novelty. Integrating age or body mass as fixed effects in the model allowed us to check that individual differences were not related to small subtle differences in morphology, body condition or health.

By comparing the behavioural responses of chipmunks in the hole-board in two situations differing in their potential risks (*i.e.* under the forest cover vs open space, Mcadam & Kramer, 1998), we expected to find faster habituation of chipmunks in the low risk environment. Chipmunks did not show any difference in their behaviour between these two situations. One explanation is that chipmunks did

not recognize the difference between the two situations, because of Plexiglas roof on the top of the hole-board and because they reacted to their immediate environment. The hole-board may also have been too stressful and may have masked the potential variation between chipmunks in the two situations. The absence of significant inter-individual variation in a test may only result from limitations of test to measure inter-individual variation. In this case, after a period of habituation, it might be possible to detect variation between two different environments. It would, however, be difficult to consider the hole-board test as a novel environment test after several replicated tests if animals get habituated to it. Finally, each chipmunk was successively tested in one and the other condition, leading to a possible overall habituation hiding the differences between the situations.

Behavioural syndromes in the Eastern chipmunk

Results from the principal component analysis indicate that some behaviours were strongly associated and indicate the presence of behavioural syndromes. For example we were not able to separate activity from exploration despite experimental and statistical precautions. This suggests a genuine association between activity and exploration in this species. Exploration probably has an important role in this species, which depends on food resources that are heterogeneously distributed through space and time. On the other hand, above ground activity in chipmunks is potentially associated with high predation risks (Bowers, Jefferson & Kuebler, 1993). This means that the conditions necessary for the maintenance of a syndrome related trade-off (see Sih *et al.*, 2004) are met in this case.

We also found that chipmunks did not differ in their habituation suggesting the existence of a behavioural carryover for activity/exploration and docility. The occurrence of a behavioural carryover implicates a limited individual phenotypic plasticity for the trait (Sih, Bell & Johnson, 2004; Sih *et al.*, 2004). Such a limited

plasticity could affect individual fitness in a stochastic and heterogeneous environment. Because individual chipmunks are all identical in their rate of habituation, the trade-off probably plays a role at the level of exploration and risk taking in a new and well known situation. Highly exploratory individuals may suffer higher risk of predation in a new situation, whereas less exploratory individuals may hardly cope in a non-stable environment and may therefore incur a time cost to adjust to new conditions. Implication of variation in the reaction to novelty for fitness, and cost-benefit of variation in temperament will need further studies.

Correlation between the BLUP values also allowed us to test for the occurrence of behavioural syndromes. In our study, we could not find significant correlations between docility and hole-board behaviours (exploration/activity; grooming-urination/defecation continuum; emotionality), suggesting the absence of behavioural syndrome between these traits. These results were unexpected given that the literature on proactive-reactive axis shows a significant relationship between boldness and exploration (Sih *et al.*, 2004). Exploration (File, 2001; Mettke-Hoffmann, Winkler & Leisler, 2002) and boldness (*sensu* Wilson *et al.*, 1994) both refer to a reaction towards a new situation, environment or object, and possibly measure the same trait or similar traits. Docility is measured as a reaction to restrain stress or human presence. Our results suggest that docility in chipmunks is not related to boldness or exploration. Using tonic immobility as a measure of response to restrain stress, Heiblum *et al.* (1998) showed no relationship between tonic immobility and exploration measured in an open-field in domestic fowls chicks (*Gallus domesticus*).

Our measures of behaviours were phenotypic measures. In the absence of genetic data, we can only conclude the potential existence of a behavioural syndrome. High heritability values have been found for activity and exploration (rabbits, Daniewski & Jezierski, 2003; great tits, Dingemanse *et al.*, 2002; mice, Gershenfeld & Paul, 1997) and for habituation (cattle, Schmutz *et al.*, 2001), indicating the

potential for a genetic basis at the origin of the individual variation observed in chipmunks and consequently the existence of a behavioural syndrome between associated traits. Environmental effects specific to a chipmunk territory, such as micro-habitat variation in predation risk, resource abundance or human perturbation may also explain individual differences in temperament. We were not able to rule out these two potential influences.

The existence of behavioural syndromes in a wild animal population can have important ecological implications. Behavioural syndromes are a type of trade-off that constraint the evolution of traits (Sih *et al.*, 2004). Our study provides further support for the existence of phenotypic and potential genetic variation in temperament traits in wild populations, and raises questions about the mechanisms responsible for the maintenance of this variation.

CHAPITRE II

ANIMAL TEMPERAMENT, REACTION TO HUMAN DISTURBANCE, AND CONSEQUENCES FOR THE STUDY OF STRESS RESPONSE OF WILDLIFE TO TOURISM EXPOSURE

ABSTRACT

Studies on the response of wildlife to human disturbance generally focus on demographic changes, or on physiological and behavioural modifications directly related to stress response. Yet no study has explored whether individual animals will distribute according to their temperament in response to human disturbance. Temperament represents the inter-individual variation in reaction to a novel, or challenging situation. Individuals are thus assumed to express highly consistent behaviour-hormonal response under specific stress conditions. In this study we investigate the relations between exploration, emotionality, and docility of individual Eastern chipmunks (Tamias striatus) and location of their burrow respective to human perturbation. We then assessed the relationship between cortisol accumulated in the hair and both temperament and human perturbation. Explorative or docile chipmunks were related with perturbed area. Long term stress response measured with cortisol hair was correlated with docility and not with human perturbation. These results indicate that as a function of their temperament animals may distribute in a non-random way according to human disturbance. They also raised the question of the interaction between temperament and human interactions on the hormonal response to stress. This study suggests that integrating temperament in the analysis of stress response of wildlife to humans could help understand the impact of tourism on wildlife.

Keywords: temperament trait, hair cortisol, tourism exposure, hole-board test.

INTRODUCTION

Watching wildlife has become a very popular recreational activity worldwide with the number of participants steadily increasing (Giannecchini, 1993). The challenge of ecotourism is to combine the demands of tourists with the needs of local populations and the conservation of protected areas (Giannecchini, 1993). Whatever forms ecotourism takes, it may have a negative impact on the physical and biological environment and on wildlife behaviour. Numerous studies have documented increased habituation, decreased feeding time, hormonal changes, increased predation, decreased survivorship or reproductive success, and changes in the social and mating system as effects of tourist activities on wildlife (*e.g.* beluga whales, Blane & Jaakson, 1994; Magellanic penguins, Fowler, 1999; Cuban rock iguana, Lacy & Martins, 2003; Asian rhinos, Lott & Mccoy, 1995; marine iguanas, Romero & Wikelski, 2002).

Studies of behavioural and physiological modification in free-ranging animals exposed to human perturbation have emerged (Fowler, 1999; Romero & Wikelski, 2002; Walker, Boersma & Wingfield, 2005), along with a growing interest in the individual based approach in conservation (Mcdougall *et al.*, 2006). Stress induced by human disturbance on animals is now widely accepted (Carney & Sydeman, 1999; Dyck & Baydack, 2004; Fowler, 1999). Yet few studies have investigated how depending on their temperament individuals could be differentially affected by and show different stress responses to human disturbance (Mcdougall *et al.*, 2006). Temperament is defined as the individual consistency in behaviours over time and across situations (Mcdougall *et al.*, 2006), and behavioural temperament differences are highly associated with specific endocrinal responses (Buss *et al.*, 1987). According to Koolhass *et al.* (1999) in response to a stress reactive (*i.e.* docile, and

lowly explorative) animals show higher cortisol release than proactive (*i.e.* active, non docile, and highly explorative) individuals. Furthermore, the exploration profile of an individual affects its potential to disperse (Dingemanse *et al.*, 2003; Fraser *et al.*, 2001). Therefore, depending on temperament, individuals within a population may vary in their potential to occupy areas varying in the level of human perturbation. For example perturbed areas may be favoured by explorative-bold, proactive, animals, and avoided by non-explorative-shy, reactive individuals. As a consequence, endocrinal differences between animals occupying disturbed and undisturbed areas may not be solely a direct effect of stress response to human perturbation, but may also reflect the non-random spatial distribution of individuals of different temperaments.

In this paper we examine the distribution across a human perturbation gradient of individual Eastern chipmunks (*Tamias striatus*) according to their temperament, and we analyze the effects of temperament and perturbation on individual stress response. The Eastern chipmunk is a charismatic rodent commonly distributed across eastern North America. In the Gault Nature Reserve (Canada), a Unesco Biosphere reserve, Eastern chipmunks have been in intense contact with tourists, and often fed by them, for several decades. In a previous study (see Chap.1), we have shown strong individual consistency in exploration, emotionality and docility in that population. Here we first investigate the relationship between chipmunks exploration, emotionality, and docility and the level of human disturbance around their burrow. We then examine the effects of temperament and human disturbance on stress response by analyzing cortisol accumulation in hair as an index of chronic stress. We finally discuss the implication of temperament and stress response for studies of the impact of human disturbance on wildlife.

METHODS

Study area and model species

Field work was conducted in the public area of the Gault Nature Reserve at Mont Saint Hilaire, Québec, Canada (45.5°N, 73.1°W), from May 2 to September 22 2004. This study area consists of mature hardwood forest dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolium*) and red oak (*Quercus rubra*).

Chipmunks are ground-dwelling, diurnal rodents (Elliott, 1978), and their period of most intense above-ground activity (April to October) corresponds to the period of highest activity by humans (Gault Nature Reserve, pers. com.). Chipmunks occupying the most perturbed zone of the Reserve can hardly avoid daily contacts with humans. Furthermore, they are habituated by the presence of people and are often fed by them. Chipmunks defend a home range with undefined boundaries. Home ranges overlap extensively, but areas close to burrows are actively defended and there is a strong space-related dominance (Elliott, 1978). Chipmunks spend more than 90% of their time in a 40-meter radius surrounding their burrow entry (Burke Da Silva, Mahan & Da Silva, 2002; Elliott, 1978). We will refer to this area as a chipmunk home range hereafter.

Chipmunks were live-trapped with Sherman traps baited with sunflower seeds. We identified the burrow of 24 chipmunks previously trapped, sexed, weighed, ear-tagged (National Band and Tag Co., New-Port, Kentucky) and fur-clipped for identification at distance (Elliott, 1978; Giraldeau *et al.*, 1994). All the chipmunks trapped and studied had their burrows within an area of 50 ha near the lake shore. Some chipmunks were already marked from studies done prior to 2004. However,

because of a lack of complete long-term information, chipmunks could not be aged precisely. We therefore considered two age categories: individuals captured prior to 2004, and individuals captured for the first time in 2004.

Human perturbation and chipmunk density

The annual number of visitors has dramatically increased during last decade to reach 170,000 in 2004 (Gault Nature Reserve, pers. comm.). The area is covered by a network of public trails. The reserve regulation, however, restricts people to using the trails. Human perturbation in a chipmunk's territory could be enhanced in two ways: a large number of trails crossing the territory and an increase in human frequentation on trails. The study area could be separated in to two zones according to the level of human frequentation. The zone of high perturbation, the most frequented by the public, includes a picnic area on a lake shore and is characterized by the highest density of trails. In the zone of low perturbation the public only uses few trails leading to viewpoints on summits. During summer and the fall, it is common to observe between 10 to 70 people present in permanence in the zone of high perturbation. Conversely, a maximum rate of 20 people per hour was observed walking on trails in the low perturbation zone, and fewer than 10 people could be observed on the trails on week days (pers. obs.). To differentiate the two zones we attributed a score of zero and a value of one to the zone of low and of high perturbation, respectively. We then calculated the number of trails crossing a chipmunk's home range. In the low perturbation zone, a maximum of one trail crossed a home range, whereas up to three trails could cross a home range in the zone of high perturbation. By summing the score of the zone and the number of trails crossing a home range, we obtained a perturbation index, ranging from zero to four, where zero represented the lowest level of perturbation (*i.e.* a chipmunk located far from a trail in the zone of low perturbation), and four represented the highest level of

potential perturbation by humans (*i.e.* a chipmunk located in the zone of high perturbation, with three trails crossing its home range).

For each chipmunk we also calculated an index of neighbour density by counting the number of home ranges that overlapped with the home range of a given chipmunk. We defined two different categories of density: lone individuals and individuals with at least one neighbour's home range overlapping with their home range (range: 1-2).

Behavioural observations

During the summer 2005, repeated hole-board tests (average number of tests per ind = 3.7; range = 2-5) and handling bag tests (average number of tests per ind = 5.7; range = 2-10) were realized to determine the temperament profil of individual chipmunks. The hole-board is a derivate of the open-field apparatus with holes in the floor (File & Wardill, 1975). The test consists of measuring the behavioural reaction of an animal in a novel environment from which escape is prevented by a surrounding wall (Walsh & Cummins, 1976). Briefly, latency to enter in the apparatus, scanning, grooming, rearing, climbing, head-dipping, ambulation, defecation and urination behaviours were measured in the hole-board. In a principal component analysis, the first component represented an exploration index and the third component an emotionality index (see Chap.1). We did not consider the second component (*i.e.* grooming-scanning continuum), which did not show any significant variation among individuals and therefore could not allow us to determine any individual profile. Measures of an individual's reaction toward human presence (Korhonen, Jauhiainen & Rekila, 2002) or to a restrain stress (Heiblum *et al.*, 1998) are often used as a docility index (also called tameness; see McDougall *et al.* 2006). Handling bag test was performed to measure behavioural response to human presence and manipulation. Rapidly after its release from a trap a chipmunk was placed in a

handling bag and the proportion of time spent immobile recorded during 1 min, as a measure of docility (see Chap. 1).

Hormonal analysis

We measured cortisol accumulation in the hair as a measure of chronic stress during the period of hair growth (Kalra *et al.*, 2005; Klein *et al.*, 2004; Koren *et al.*, 2002; Raul *et al.*, 2004). Hair was collected by fur-clipping the rump of 23 individuals in May-June and in August-September, after the two molting peaks (Snyder, 1982). Six chipmunks fur-clipped in September 2003 after molting could be fur-clipped again in May and September 2004. This observation suggests that each individual molted at each molting peaks. Cortisol in hair represented an overall accumulation during hair growth (Koren *et al.*, 2002; Yang *et al.*, 1998). Therefore hair sampled in May-June (*i.e.* spring hair) represented cortisol accumulation in hair during April-May, when chipmunk above-ground activity and frequentation by tourists of the reserve were low. Hair sampled in August-September (*i.e.* summer hair) represented cortisol accumulation in hair when chipmunk above-ground activity and frequentation by tourists were high.

For extraction we used the Yang *et al.* (1998) protocol. For each animal, 15-40 mg of hair was cut into 3-4 mm pieces. Hair pieces were mixed with 2 ml of ether in a test tube, vortexed for one minute, and then left for one hour. The extracted solution was transferred into another test tube. One ml of ether was added into the original test tube and the above step was repeated. The two solutions were mixed and dried with a vacuum pump. Each sample was reconstituted with 0.1 ml of phosphate buffer solution (PBS PH=7.0), and was ready for the assay.

Each sample was dispensed into the appropriate polyclonal hormone antiserum coated well of the ELISA (solid phase enzyme linked immunoassay)

microplate, in duplicates. We measured the samples for hormones, using Cortisol Correlate-EIA™ kits from Assay Designs (Ann Arbor, MI, USA), according to the manufacturer's protocols. Briefly, conjugate and antiboby were added to each well and the microplate was incubated for two hours at room temperature, on a plate shaker at 50 rpm. Microplate was then washed, substrate solution added, and the plate incubated one hour without shaking. The absorbance was read at 405nm just after adding stop solution. From the standard curve, we calculated the concentration of hormone in samples first in pg/ml and then transformed it into pg/mg hair. All standards and reagents were provided by the kit.

Statistical analyses

Individual behavioural profile

Linear mixed models of behaviours were performed using date, hour, trial order, sex, capture year, and weight as fixed effects and chipmunk identity as a random effect. Best linear unbiased predictors (BLUPs) for random effects were used as predictors of individual behaviour profiles. BLUP method provides prediction of random effects independent of other terms within a model, standardized to a mean of zero (Kruuk, 2004). They also have the advantage of being less sensitive to extreme values within data than separate regression estimates (Pinheiro & Bates, 2000). Therefore BLUPs provide more appropriate estimates of the behaviour profile of an individual than the mean of all the measures for that individual.

Impact of tourism

Impact of tourism exposure on behaviours was evaluated using a linear regression on individual behavioural profile as a function of perturbation index and

chipmunks density. Cortisol in hair was log-transformed to normalize the data. We compared hair cortisol levels for the same animals sampled in May-June and in August-September using a paired t-test. Linear regression on hair cortisol as a function of behavioural indices, chipmunk density and perturbation were assessed for the two sampling periods. Statistical analyses were performed with R 2.2.0 (Ihaka & Gentleman, 1996).

RESULTS

Exploration and docility were positively and significantly related to perturbation index (Table 2.1). Chipmunks with a burrow in highly perturbed areas showed higher exploration scores in the hole-board and moved less in handling bag (*i.e* higher docility) than chipmunks located in lowly perturbed areas (Fig. 2.1). Emotionality was not significantly related to the perturbation index. Chipmunk density had no significant effect on the three temperament traits (Table 2.1).

Hair cortisol concentration was significantly higher in August-September than in May-June samples (paired t-test: $t_7 = -2.61$, $P = 0.034$) (Fig. 2.2). None of the temperament variables, age, perturbation index or chipmunk density affected May-June hair cortisol (Table 2.2). Cortisol concentration in summer decreased significantly with age and increased significantly with docility (Table 2.2 and Fig. 2.3), but was not significantly affected by exploration and emotionality, or by human perturbation and chipmunks density. Removing temperament data from the model did not change the relationship between cortisol and both perturbation ($P = 0.49$) and chipmunk density ($P = 0.90$).

Table 2.1 Estimates of environmental effects in linear model of chipmunks behaviours.

	Exploration			Emotionality			Docility		
	Estimate	Test statistic	*P*	Estimate	Test statistic	*P*	Estimate	Test statistic	*P*
(Intercept)	-0.67 ± 0.29			-0.06 ± 0.18			-0.26 ± 0.16		
Perturbation index	0.30 ± 0.13	$F_{1,21} = 5.46$	**0.029**	0.05 ± 0.09	$F_{1,21} = 0.01$	0.956	0.19 ± 0.08	$F_{1,21} = 6.12$	**0.021**
Chipmunk density	0.29 ± 0.34	$F_{1,21} = 0.73$	0.399	-0.07 ± 0.22	$F_{1,21} = 0.08$	0.780	0.02 ± 0.19	$F_{1,21} = 0.01$	0.915

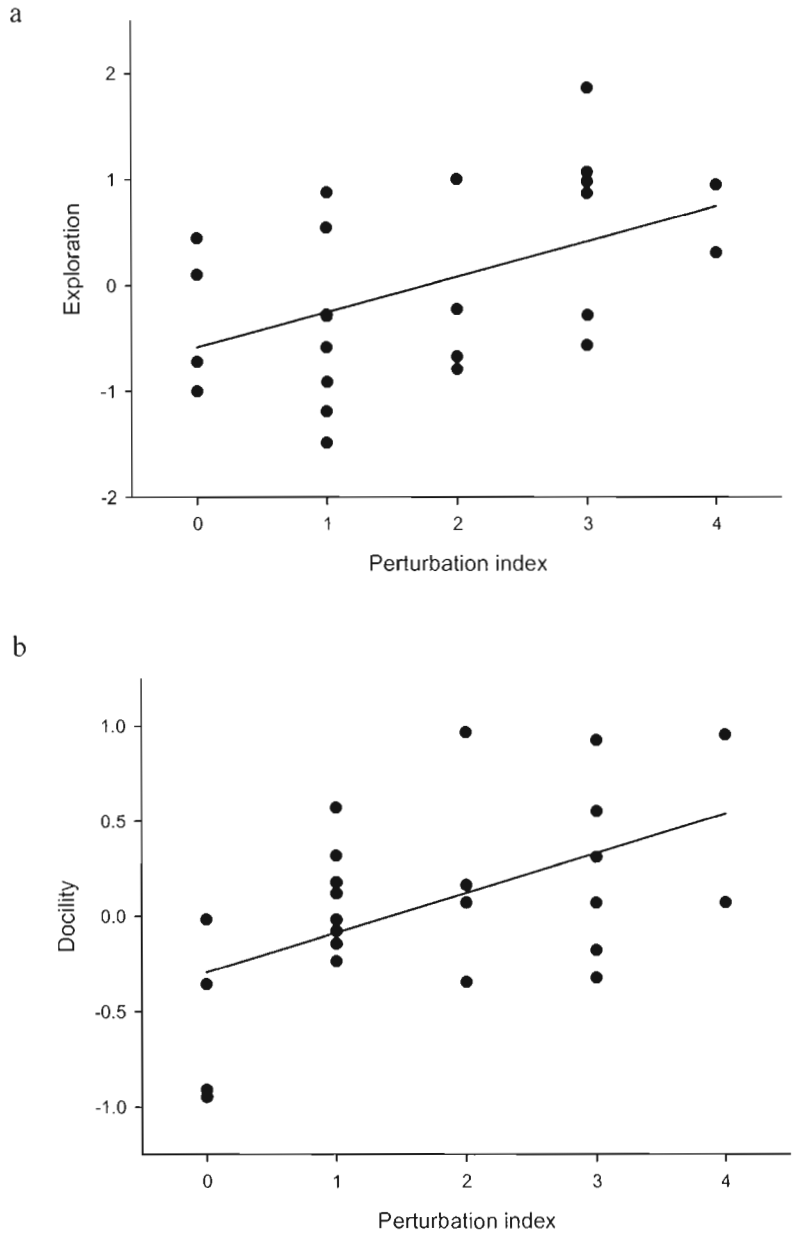

Figure 2.1 Chipmunks' exploration (a) and docility (b) with human perturbation. Each dot represents an individual. Human perturbation was measured using human frequentation and number of trails crossing a chipmunks' territory. Individual values of behaviours are provided by the Best Linear Unbiased Predictors of exploration and docility.

Table 2.2 Estimates of environmental and behavioural effects in a linear model of Eastern chipmunk's hair cortisol concentration

	Spring hair cortisol concentration			Summer hair cortisol concentration		
	Estimate	Test statistic	*P*	Estimate	Test statistic	*P*
(Intercept)	0.71 ± 0.39			3.46 ± 0.52		
First capture year	-0.07 ± 0.26	$F_{1,8} = 0.07$	0.800	0.91 ± 0.34	$F_{1,9} = 7.19$	**0.025**
Perturbation index	-0.01 ± 0.17	$F_{1,8} = 0.01$	0.988	-0.03 ± 0.14	$F_{1,9} = 0.06$	0.817
Chipmunk density	0.42 ± 0.30	$F_{1,8} = 2.04$	0.191	-0.09 ± 0.33	$F_{1,9} = 0.07$	0.791
Docility	0.18 ± 0.27	$F_{1,8} = 0.45$	0.521	1.01 ± 0.40	$F_{1,9} = 6.29$	**0.033**
Exploration	0.03 ± 0.20	$F_{1,8} = 0.03$	0.880	-0.09 ± 0.18	$F_{1,9} = 0.25$	0.626
Emotionality	-0.46 ± 0.28	$F_{1,8} = 2.68$	0.14	-0.33 ± 0.27	$F_{1,9} = 1.57$	0.242

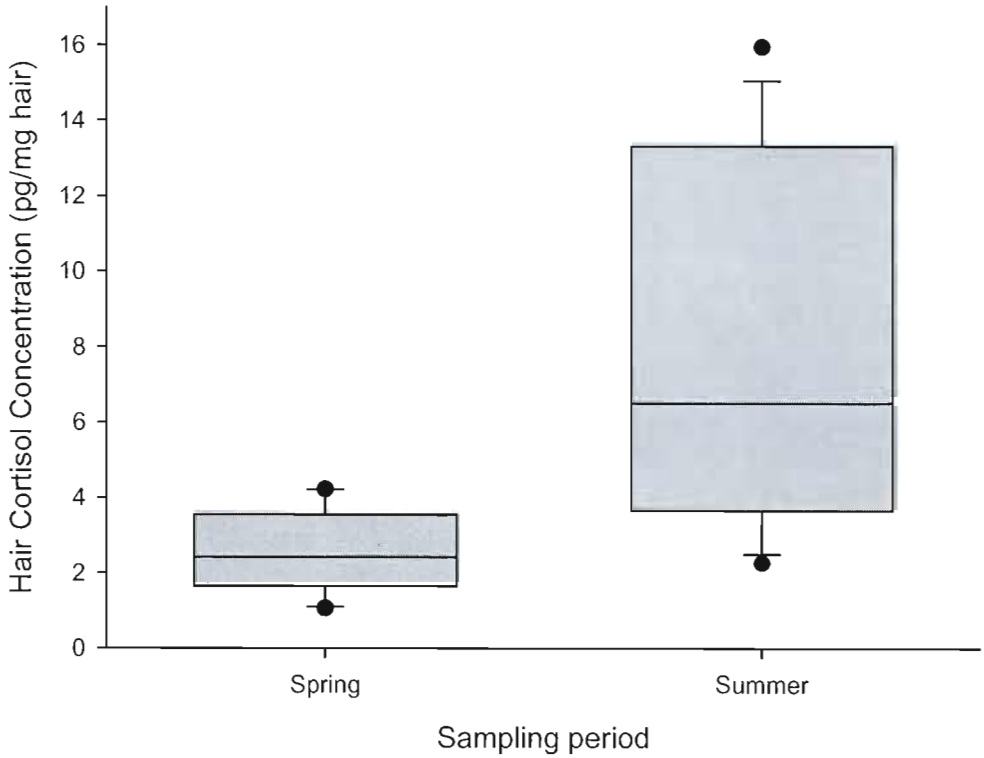

Figure 2.2 Hair cortisol concentration of Eastern chipmunk during spring and summer 2004 at Mont-Saint-Hilaire, Quebec. Hair was sampled after molting peaks in may-june and august-september. Cortisol in hair is used as an index of chronic stress.

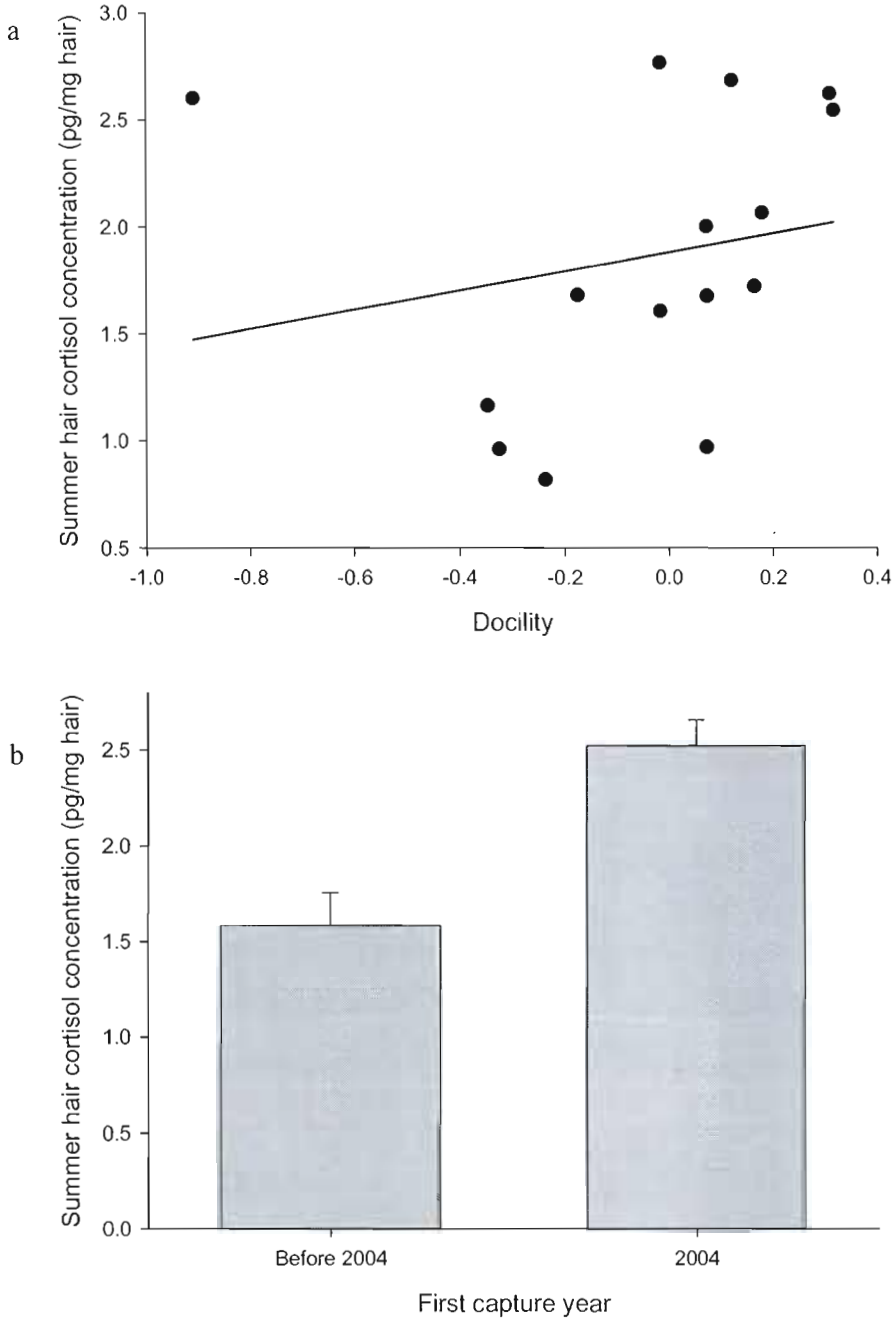

Figure 2.3 Variation of (log-transformed) summer hair cortisol with docility (a) and age (b). (a) Regression of hair cortisol as a result of docility. Each dot represents an individual. (b) Mean value of summer hair cortisol for each age category with standard error. Age categories are based on year of first capture of the individual.

DISCUSSION

Behavioural observations

Chipmunks were not distributed at random with human perturbation; more explorative and more docile individuals occupied home ranges in the most perturbed area. This non-random distribution could be caused by two possible reasons: 1) chipmunks could occupy areas varying in their level of perturbation because of their intrinsic differences in temperament, and 2) chipmunks could show different temperaments because they experienced different levels of human perturbation during ontogeny. According to the first hypothesis an animal could select its home range according to its temperament. Dispersal and habitat selection for an individual are influenced by its propensity to explore (Dingemanse *et al.*, 2003; Fraser *et al.*, 2001). Temperament traits such as exploration or docility often show significant heritabilities (*i.e.* ranging from 0.22 to 0.41, Dingemanse *et al.*, 2002; Gershenfeld & Paul, 1997; see also Koolhass *et al.*, 1999). This means that strong inter-individual variation in temperament traits has a genetic basis despite a potential plasticity (*i.e.* an individual can modulate its temperament by learning and habituation). With the second hypothesis a chipmunk that settles in a perturbed area could get habituated to humans and become more explorative and more docile than a chipmunk that settles in an undisturbed area. In a previous study (see Chap. 1) we have found that whatever their location chipmunks decreased docility when they were repeatedly tested in the handling bag. This result suggests that contrary to this first hypothSesis docility should decrease in areas highly frequented by humans. It is worth noting that our individual docility profile (*i.e.* the BLUP of docility) was corrected for the effect of repeated handling bag test, and therefore the variation in docility among chipmunks could not be caused by the difference in the number of tests they experienced. Ruling

out these two explanations is difficult without examining temperament and dispersal patterns of individuals marked as juveniles on their natal home range, and further studies will be needed. However, whatever the causes of this relation between temperament and human disturbance, the existence of such a distribution of temperament with perturbation could have important consequences on conservation studies (see below and Mcdougall *et al.*, 2006).

Hormonal measures

Cortisol concentration increased in summer hair compared to spring hair. Spring hair cortisol concentration was not linked to any environmental or behavioural variables. Low mean and variance in cortisol level in spring hair limits the power of our test to detect potential effects. However a most parsimonious explanation is that chipmunks experienced less perturbation during early spring than during summer. Chipmunks emerge from hibernation by the end of April-early May and spend most of their time in their burrow before that period (Elliott, 1978). High summer above-ground activity in chipmunks is probably related to a higher exposition to stressful situations such as predator attacks, conspecific aggression or human perturbation during that period. Human perturbation did not affect summer hair cortisol level, suggesting that it was not the main factor responsible for the stress of chipmunks, and other factors such as intra-specific aggression, predation, or captures and manipulations might play a role in cortisol secretion. Increase in hair cortisol in summer could also reflect an intrinsic seasonal variation in glucocorticosteroids in chipmunks (Kenagy & Place, 2000).

Our results did not permit us to say that chipmunks in perturbed areas were more stressed than those located in zones of low perturbation. The absence of relation between human perturbation and hair cortisol could result from a reduced cortisol release in chipmunks occupying frequented areas following their habituation to

human perturbation (Fowler, 1999; Romero & Wikelski, 2002). Studies of short-term cortisol release in the blood as a response to human presence would be needed to distinguish those different explanations. However, these techniques are invasive and may not be recommended for someone who is undertaking a survey of populations for conservation purpose.

Summer hair cortisol concentration was positively related with docility suggesting the existence of different coping styles (*sensu* Koolhass *et al.*, 1999) in chipmunks. However, contrary to Koolhass *et al.* (1999) we could not find any relationship between docility and exploration, even after adjusting for the effect of human perturbation. Such association between temperament and cortisol or corticosterone release has been found in several species (birds, Carere *et al.*, 2003; monkeys, Cavigelli, 1999; rodents, Koolhass *et al.*, 1999; Sgoifo *et al.*, 1996; Veenema *et al.*, 2003) indicating the importance of integrating temperament variables when comparing hormonal differences in studies of stress response on human perturbation.

Chipmunks captured for the first time in 2004, potentially juveniles or new immigrants in the study area, showed higher cortisol accumulation in the hair than those captured in previous years. Thus the effect of first capture on cortisol accumulation could be interpreted as an age effect or an effect of habituation to human disturbance or to other stressful situations such as predation, but the exact mechanisms of habituation are unknown (Romero & Wikelski, 2002). The effect of capture and manipulation on chipmunks behaviour and cortisol could be rejected because we could not find any significant relationship between the number of captures of a chipmunk and cortisol in the hair, or behaviours (all $r < 0.40$, $p > 0.20$).

Conservation implications

Using an individual based method, we adopted an original approach to study the impact of human disturbance on wildlife and we pointed out an important problem: according to their temperament, individuals are not randomly distributed in a human perturbation gradient. Ignoring variation in temperament when analyzing the stress response of wildlife to human disturbance may thus possibly lead to biased conclusions. This could happen especially when comparing two groups originating from a perturbed and an undisturbed area, like most of studies on human impact. Endocrinal response of each of these groups may result from specific stress responses associated with the temperament of individuals occupying the different areas. In absence of knowledge on animal temperament tourism impact could be under- or overestimated. Animal temperament may thus be considered in future studies on human impact on wildlife.

CONCLUSION GÉNÉRALE

Nous avons développé une méthode de mesure du tempérament réalisable directement sur le terrain et permettant de tester à la fois la variabilité des comportements et de l'habituation. Cette méthode est une approche basée sur l'utilisation de modèle mixte dans l'analyse de tests du hole-board et du sac de capture réalisé de manière répétés sur les mêmes individus, mais pourrait s'appliquer à d'autre tests de tempérament, comme le test du nouvel objet ou les tests de confrontation agonistique (test de miroir; test de rencontre dyadique). Avec cette méthode, nous avons mis en évidence : 1) une variation individuelle dans la réponse comportementale à une nouvelle situation; 2) une habituation aux situations expérimentales; 3) un transfert comportemental des individus avec une diminution de la nouveauté (c'est-à-dire le fait que les individus maintiennent un rang stable dans leur valeur de comportements au cours du temps, malgré un changement globale du comportement dans la population dû à l'habituation). Nous n'avons pas montré de relation entre les différents traits de tempéraments mesurés (exploration/activité, toilettage-excrétion, émotivité et docilité) impliquant une absence de syndromes comportementaux entre ces traits. À partir des valeurs individuelles de comportement, nous avons testé l'effet de la perturbation humaine sur le tempérament et la réponse physiologique à cette perturbation. Nous avons montré une distribution non-aléatoire des individus en fonction de leurs traits de tempérament et du niveau de perturbation humaine. Les tamias explorateurs ou moins farouches étaient présents dans les zones perturbées. Enfin, nous avons montré que la réponse physiologique au stress, c'est-à-dire concentration de cortisol mesurée dans les poils et dans les crottes, était affectée par le tempérament des individus mais ne dépendait pas du degré de perturbation humaine.

Les mesures de tempérament sur les animaux sauvages réalisées directement sur le terrain ont plusieurs avantages par rapport aux mesures en laboratoire. Le premier avantage, et vraisemblablement le plus important dans un programme de recherche sur l'écologie du tempérament, est que les mesures de tempérament sur le terrain limitent les effets secondaires potentiels causés par le transport, la captivité et le relâchement des animaux dans leur habitat après l'expérience. L'ensemble de ces manipulations pourrait affecter l'écologie et les comportements des animaux étudiés. Les observations comportementales d'animaux en milieu sauvage permettent difficilement de mesurer la variation interindividuelle de tempérament. Celle-ci est souvent exprimé dans une situation risquée ou nouvelle, et ces situations sont difficiles à observées en grand nombre sur le terrain. Une approche expérimentale et éthologique est alors recommandée, car elle permet de confronter les individus à une même situation stressante et de réaliser le nombre d'observation souhaité. Nos résultats montrent qu'il est possible d'utiliser une telle approche pour étudier les traits de tempérament ou de personnalité sur le terrain. L'ensemble de nos procédures de test durait 30 minutes environ entre la capture et le relâchement de l'individu. Les tests de hole-board, d'open-field ou du sac de capture sont faciles et rapides à réaliser même sur le terrain. Ils permettent de collecter des données sur un grand nombre d'individus, nombre nécessaire pour les programmes en écologie évolutive. Cependant, ces tests peuvent difficilement être utilisés sur des grands animaux (ex : ours, cerfs) ou sur des espèces très mobiles (ex : belette, hermine) qui nécessiteraient des arènes de trop grande taille donc peu facilement transportables. De plus, notre méthode nécessite un grand nombre de captures pour obtenir l'ensemble des répliquas nécessaires. Avec des espèces difficiles à capturer ou présentes pendant un court laps de temps sur une aire d'étude, les tests en laboratoire pourraient être avantageux puisqu'une seule capture serait nécessaire pour réaliser l'ensemble des tests souhaités avant de relâcher l'animal.

Outre les mesures de terrain, nous préconisons l'utilisation de modèles mixtes dans l'étude du tempérament et du comportement animal. Utilisé avec des mesures individuelles répétées, l'approche du modèle mixte a l'avantage de combiner plusieurs objectifs en une seule analyse robuste. Les modèles mixtes permettent d'examiner à la fois la réaction des individus à la nouveauté et à la diminution de la nouveauté lorsque les tests sont répétés. La comparaison des pentes individuelles en fonction de l'ordre des tests permet également d'évaluer l'existence d'un transfert comportemental dans un contexte d'habituation. Finalement, les modèles mixtes donnent des prédictions des profils comportementaux individuels. En considérant l'identité des individus comme un effet aléatoire, il est possible d'obtenir une valeur prédite pour chaque comportement de chaque individu (BLUPs), indépendante des autres effets intégrés dans le modèle. Les valeurs prédites ne sont pas affectées par les biais potentiels dus à aux effets environnementaux.

Les traits de tempérament peuvent affecter la manière dont les individus interagissent avec leur environnement et avoir des effets sur leur aptitude adaptative. Par exemple, chez les mésanges charbonnières, l'exploration affecte plusieurs composantes de l'aptitude dont la survie annuelle des adultes et la production de jeunes. Deux points nous semblent particulièrement intéressants à étudier dans le futur : 1) tester la signification écologique du tempérament mesuré dans le hole-board, autrement dit, un animal considéré comme explorateur à la suite du test est-il explorateur dans son milieu ? ; 2) observer comment le tempérament influence les interactions avec l'environnement et comment il affecte l'aptitude phénotypique chez le tamia, c'est-à-dire un animal très explorateur réagit-il différemment à son environnement qu'un animal qui l'est moins et être explorateur procure t'il un bénéfice ou un coût à l'aptitude phénotypique (ex : survie, succès reproducteur) ?

L'existence de syndromes comportementaux dans une population sauvage a des implications écologiques importantes. Les syndromes comportementaux sont une

sorte de compromis qui limitent et contraignent l'évolution des traits. Nous avons mis en évidence un syndrome comportemental potentiel caractérisé par une corrélation phénotypique entre l'activité et l'exploration ainsi qu'un transfert comportemental individuel avec une diminution de l'habituation. Étant positivement liées, l'activité et l'exploration devraient co-évoluer, suite au compromis entre l'avantage qu'offre l'exploration pour la découverte de nouvelles ressources, et le coût associé à une forte activité par la prédation. Par ailleurs, si notre absence de variation interindividuelle au niveau phénotypique traduit une absence de variation au niveau génétique pour l'habituation, nous pouvons considérer que le potentiel d'évolution de la capacité d'habituation pourrait être limité dans cette population de tamias. D'autres travaux seraient nécessaires pour évaluer la relation au niveau génétique. Cependant ces travaux n'étaient pas possibles durant le temps d'une maîtrise. Il nous aurait alors fallu disposer de l'information complète sur le pedigree, et des mesures de tempérament, pour un grand nombre d'individus dans la population. En premier indice nous considérons que la relation phénotypique est un bon indicateur.

Certaines de nos analyses sur les réponses hormonales à la perturbation humaine peuvent manquer de puissance statistique, étant donné la faible taille de l'échantillon utilisé (13 tamias pour le niveau de cortisol dans les poils). Cependant, certains résultats comme la distribution spatiale des tamias en fonction de leur tempérament et de la perturbation, la variation saisonnière de cortisol, ou la relation positive entre cortisol et tempérament, sont apparus significatifs malgré ce petit échantillon. Ces résultats semblent suffisamment originaux pour mériter d'être mentionnés : ils mettent en évidence un problème important dans les études d'impact de la perturbation humaine sur la faune sauvage. Les tamias les plus explorateurs et les moins farouches étaient localisés dans les zones de perturbation humaine élevées et les taux hormonaux étaient affectés par le tempérament des individus mais pas par le niveau de perturbation. La répartition non-aléatoire du tempérament selon la perturbation pourrait engendrer une différence comportementale et hormonale entre

les zones perturbées et non-perturbées qui ne serait pas directement due à la perturbation. L'absence de connaissance sur le tempérament des animaux peut entraîner une sous- ou une surestimation de l'impact du tourisme sur la vie sauvage. L'existence d'un biais dans l'estimation de l'impact de la perturbation est d'autant plus probable lorsque deux groupes d'animaux provenant de zones perturbées et non perturbées sont comparés comme le font la majorité des études sur la perturbation humaine. Il serait bon d'intégrer le tempérament animal dans les prochaines études sur l'impact de l'homme sur la faune sauvage.

Nous avons utilisé des mesures non-invasives de cortisol dans les poils et dans les crottes comme indice du stress chez les tamias. La manipulation des animaux étant elle-même stressantes, l'utilisation de méthodes non-invasives pour mesurer le taux de cortisol diminue le biais créé par le stress de la manipulation. Cependant, ces méthodes ne permettent pas de mesurer la réponse hormonale immédiate à un stimulus stressant. Hors, il serait intéressant de mesurer la réponse hormonale au test du hole-board et à la perturbation humaine. Cette mesure permettrait entre autres de tester les relations entre tempérament et hormones, valider les syndromes comportementaux à un niveau physiologique et confirmer l'effet du tempérament dans les études sur la perturbation humaine.

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